



GREENBLUM & BERNSTEIN, P.L.C.
Intellectual Property Causes
1950 Roland Clarke Place
Reston, VA 20191
(703) 716-1191

TW
AF

In re application of : Masatoshi CHIBA

Mail Stop Appeal Brief-Patents
Confirmation No. 5687
Attorney Docket No. P21749

Application No. : 09/926,661

Group Art Unit : 1649

Filed : February 28, 2002

Examiner : D. E. Kolker

For : LYOPHILIZED HGF PREPARATIONS

Mail Stop Appeal Brief-Patents

Commissioner for Patents

U.S. Patent and Trademark Office

Customer Service Window, Mail Stop Appeal Brief-Patents

Randolph Building

401 Dulany Street

Alexandria, VA 22314

Sir:

Transmitted herewith is an **Amendment Appeal Brief Under 37C.F.R. §41.37(d)** in the above-captioned application.

☐ Small Entity Status of this application under 37 C.F.R. 1.9 and 1.27 has been established by a previously filed statement.

☐ A Request for Extension of Time.

☒ Evidence Appendices: Copies of EP 0 456 188; WO 97/02832; US 2001-0051604; *Ex Parte* Bobsein et al., (Appeal No. 2005-1332); *In re* Arkley, 172 U.S.P.Q. 524 (CCPA 1972).

☒ No additional fee is required.

The fee has been calculated as shown below:

Claims After Amendment	No. Claims Previously Paid For	Present Extra	Small Entity		Other Than A Small Entity	
			Rate	Fee	Rate	Fee
Total Claims: 20	*21	0	x25=	\$	x 50=	\$ 0.00
Indep. Claims: 2	**4	0	x100=	\$	x200=	\$ 0.00
Multiple Dependent Claims Presented			+180=	\$	+360=	\$ 0.00
Extension Fees for ___ Month(s)				\$		\$ 0.00
Total:				\$	Total:	\$ 0.00

* If less than 20, write 20

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☐ Please charge my Deposit Account No. 19-0089 in the amount of \$ ____.

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☒ Any additional filing fees required under 37 C.F.R. 1.16.

☒ Any patent application processing fees under 37 C.F.R. 1.17, including any required extension of time fees in any concurrent or future reply requiring a petition for extension of time for its timely submission (37 C.F.R. 1.136(a)(3)).

Sean Myers Payne
Bruce H. Bernstein
Reg. No. 29,027
42,920



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellant : Masatoshi CHIBA

Group Art Unit: 1649

Serial No : 09/926,661

Examiner: Daniel E. KOLKER

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AMENDED APPEAL BRIEF UNDER 37 C.F.R. § 41.37(d)

Further to the Notification of Non-Compliant Appeal Brief mailed January 18, 2007 and to the Notice of Panel Decision from Pre-Appeal Brief Review mailed August 18, 2006, this Appeal Brief is responsive to the final Office Action mailed February 16, 2006 and to the Advisory Action mailed June 8, 2006. Inasmuch as the Notification sets a one-month shortened statutory period for response, this Amended Appeal Brief is timely filed.

Appellant respectfully notes that the previous Appeal Brief is compliant, as it complies with the rules by providing the contentions of Appellant with respect to each point of rejection presented for review, as required by paragraph 37 C.F.R. § 41.37(c)(1)(vi), and the basis therefor with supporting citations where appropriate. Moreover, the Rules do not require that the Evidence Appendix list the documents relied upon by the Examiner in the rejections. However, in order to advance prosecution, Appellant has amended the Appeal Brief to specifically identify the statutes relied upon by the Examiner and to list and provide copies of documents relied upon by the Examiner.

If any additional fees are due for consideration of this Amended Brief, including any extension of time fees, the Office is authorized to charge such fees to Deposit Account No. 19-0089.

Application No. 09/926,661

Attorney Docket No. P21749

Amended Appeal Brief Under 37 C.F.R. § 41.37(d)

I. Real Party In Interest

The assignee, Mitsubishi Chemical Corporation, is the real party in interest.

Application No. 09/926,661
Attorney Docket No. P21749
Amended Appeal Brief Under 37 C.F.R. § 41.37(d)

II. Related Appeals and Interferences

None.

III. Status of Claims

Claims 1, 3, 4, 6-16, and 22-28 are pending in this application.

Claims 2, 5, and 17-21 have been canceled.

Claims 22-28 stand withdrawn from consideration as directed to a non-elected invention, pursuant to the restriction requirement made by the Examiner in the Office Action mailed June 28, 2005, and Appellant's election made in the communication dated July 28, 2005. An election of species requirement further required Appellant to choose a species from arginine, lysine, histidine, glutamine, proline, glutamic acid, aspartic acid, and sulfated polysaccharides. Appellant elected arginine.

Claims 1, 3, 4, and 6-16 stand finally rejected. Appellant appeals the rejection of claims 1, 3, 4, and 6-16.

IV. Status of Amendments

There are no amendments that have not been entered. The claims are in their form as amended in the Amendment under 37 C.F.R. § 1.116, filed May 16, 2006, entry of which was indicated by the Examiner in the Advisory Action mailed June 8, 2006. The Advisory Action withdrew the rejection of claim 11 under 35 U.S.C. § 112, second paragraph, but maintained the rejections under 35 U.S.C. §§ 102(b) and 103(a).

V. Summary of Claimed Subject Matter

The following description is made with respect to the independent claims and includes reference to particular parts of the specification. As such, the following is merely exemplary and is not a surrender of other aspects of the present invention that are also enabled by the present specification and that are directed to equivalent structures or methods within the scope of the claims.

Independent claim 1 relates to a lyophilized preparation comprising a hepatocyte growth factor (specification, page 4, lines 9-10), a stabilizing agent comprising arginine, lysine, histidine, glutamine, proline, glutamic acid, or aspartic acid, or a pharmacologically acceptable salt thereof (specification, page 5, lines 12-14), for preventing formation of an aggregate of the hepatocyte growth factor (specification, page 4, line 10), sodium chloride (specification, page 4, lines 10-11), and a buffering agent (specification, page 4, line 11), which is prepared from an aqueous solution containing the hepatocyte growth factor at a concentration lower than 5 mg/mL (specification, page 4, line 12).

Independent claim 3 relates to a lyophilized preparation comprising a hepatocyte growth factor (specification, page 4, lines 9-10), a stabilizing agent comprising arginine, lysine, histidine, glutamine, proline, glutamic acid, or aspartic acid, or a pharmacologically acceptable salt thereof (specification, page 5, lines 12-14), for preventing formation of an aggregate of the hepatocyte growth factor (specification, page 4, line 10), sodium chloride (specification, page 4, lines 10-11), and a buffering agent (specification, page 4, line 11), which is prepared from an aqueous solution containing the hepatocyte growth factor at a concentration lower than 5 mg/mL (specification, page 4, line 12), and is capable of preparing an aqueous solution containing the hepatocyte growth factor at a concentration lower than 5 mg/mL by redissolution (specification, page 4, lines 15-17).

VI. Grounds of Rejection to be Reviewed on Appeal

A) Whether claims 1, 3, 4, 6-9, and 12-15 are anticipated under 35 U.S.C. § 102(b) by Nakamura et al. (European Application No. 0456188 A1), hereinafter “Nakamura”

B) Whether claims 1 and 16 are anticipated under 35 U.S.C. § 102(b) by, or in the alternative, obvious under 35 U.S.C. § 103(a) over, Nakamura

C) Whether claims 1, 3, 4, and 6-16 are obvious under 35 U.S.C. § 103(a) over Nakamura in view of Tanaka et al. (WO 97/02832), hereinafter “Tanaka”

VII. Argument

A) Whether claims 1, 3, 4, 6-9, and 12-15 are anticipated under 35 U.S.C. § 102(b) by Nakamura (European Application No. 0456188 A1).

1) Rejection of Claims 1 and 3

Initially, Appellant respectfully notes that independent claim 1 is directed to a lyophilized preparation comprising a hepatocyte growth factor, a stabilizing agent comprising arginine, lysine, histidine, glutamine, proline, glutamic acid, or aspartic acid, or a pharmacologically acceptable salt thereof, for preventing formation of an aggregate of the hepatocyte growth factor, sodium chloride, and a buffering agent, which is prepared from an aqueous solution containing the hepatocyte growth factor at a concentration lower than 5 mg/mL.

Appellant's independent claim 3 is directed to a lyophilized preparation comprising a hepatocyte growth factor, a stabilizing agent comprising arginine, lysine, histidine, glutamine, proline, glutamic acid, or aspartic acid, or a pharmacologically acceptable salt thereof, for preventing formation of an aggregate of the hepatocyte growth factor, sodium chloride, and a buffering agent, which is prepared from an aqueous solution containing the hepatocyte growth factor at a concentration lower than 5 mg/mL, and capable of preparing an aqueous solution containing the hepatocyte growth factor at a concentration lower than 5 mg/mL by redissolution.

Appellant also notes that in response to a Restriction Requirement mailed June 28, 2005, Appellant elected arginine as the specie of stabilizing agent, for purposes of examination. The claim has not been amended to remove non-elected species, in expectation of allowable subject matter.

Prior to addressing the art-based rejections, some discussion of the context of the invention is useful. Appellant respectfully notes that aqueous hepatocyte growth factor (HGF) preparations rapidly decrease in solubility of HGF at neutral pH and have the problems of aggregation, cloudiness, and gelation when stored at a low temperature or room temperature for several days. (Specification, page 2, lines 10-12.) Aqueous preparations have low

physicochemical stability, for example, forming degradation products and aggregates, and also have poor stability as a pharmaceutical preparation, for example, exhibiting reduced biological activity. (Specification, page 2, lines 13-15.) Therefore, aqueous preparations are not suitable for a long-term storage from a viewpoint of biological activity. (Specification, page 2, lines 15-16.) For at least these reasons, there is a need in the art for a lyophilized HGF preparation.

Japanese Patent Unexamined Publication No. 9-25241 discloses a lyophilized preparation of HGF (TCF) that is said to be stable over a long period, which is provided by using citrate as a buffering agent, and glycine, alanine, sorbitol, mannitol, or the like, as a stabilizing agent. (Specification, page 2, lines 22-27.) However, due to the citric acid used as a buffering agent in the lyophilized preparation, the pH of a redissolved preparation will be acidic, resulting in a solution with a high osmotic pressure, which causes problems of pain on administration by injection, or an inflammatory reaction and hemolysis at the administration site. (Specification, page 2, lines 28-31.)

Additional difficulties are presented because HGF is a substance having extremely potent physiological activities, and thus, when used as a medicament, it needs to be provided in a very low concentration. (Specification, page 2, line 32 – page 3, line 1.) Studies by the present inventors have revealed that, as for the lyophilized HGF (TCF) preparation comprising glycine or alanine described in Japanese Patent Unexamined Publication No. 9-25241 (mentioned above), little formation of aggregate was observed during storage when the lyophilized preparation was produced from an aqueous solution containing HGF at a high concentration, while aggregate formation was observed during storage when a preparation was produced in the presence of glycine or alanine by lyophilizing an aqueous solution containing HGF at a low concentration. (Specification, page 2, lines 1-10.) Thus, glycine or alanine appears to be useful as a stabilizing agent when HGF is lyophilized at a high concentration, but not when HGF is lyophilized at a low concentration. (Specification, page 2, lines 10-14.)

The present invention is thus directed at producing a lyophilized preparation that hardly forms aggregates and has excellent stability in long-term storage by using an aqueous solution containing HGF at a low concentration. It has been surprisingly discovered by the inventors that

the specific combination of the features of Appellant's claims has the effect to avoid the formation of aggregates.

Turning to the rejection, Appellant notes that Nakamura includes a broad disclosure, which does not teach or suggest Appellant's recited combination of features as a lyophilized preparation, its manner of production or its use. Nakamura broadly discloses that the therapeutic agents of his invention are generally formed into injections containing HGF solely or combined with carriers, etc. known per se. For example, he discloses that injections can be prepared by dissolving HGF in suitable buffers, followed by sterilization by filtration through a filter.

Nakamura further discloses that the therapeutic agents for hepatocirrhosis of his invention may contain other additives such as stabilizers, excipients, dissolution-promoters, adsorption-preventors, and antioxidants, and examples thereof include, for example, sugars such as mannitol and glucose, amino acids such as glycine, alanine, lysine, and arginine, proteins such as albumin, alcohols such as ethylene glycol and glycerol, hydrophilic polymers such as polyethylene glycol, inorganic salts such as NaCl, organic salts such as sodium citrate, surfactants such as Polysorbate 80 and reducing agents containing sulfur, which may be used alone or in combination. However, there is no teaching or suggestion to combine such broad disclosure of Nakamura in the manner recited in Appellant's claims.

For example, the Examples disclosed in Nakamura include Examples 1 – 5 of freeze-dried HGF preparations. However, in Examples 1 and 2, the buffer solution has a pH value of 7.4, while no amino acid is used for stabilization. In Examples 3 and 4, the aqueous solution does not contain a buffering agent. Only Example 5 discloses lyophilization of HGF with a solution comprising an amino acid (glycine). However, the solution of Example 5 is not buffered and does not contain a salt.

Appellant notes that for anticipation to exist, Nakamura must clearly and unequivocally disclose the claimed subject matter without any need for picking, choosing, or combining various disclosures. For anticipation to exist, one must not be required to pick and choose from the disclosure of Nakamura and combine them as Appellant has. Rather, for anticipation to exist, Nakamura must clearly and unequivocally disclose all of the elements of Appellant's claimed

subject matter *with sufficient specificity*. Appellant's claims are not disclosed with sufficient specificity in Nakamura to constitute anticipation of Appellant's claimed subject matter.

Appellant respectfully directs the Board's attention to the Board's decision in *Ex parte* Bobsein et al., (Appeal No. 2005-1332), and to *In re Arkley*, 172 U.S.P.Q. 524 (CCPA 1972), which is cited in *Ex parte* Bobsein, copies of both of which are attached. Those decisions stand for the proposition that, for anticipation to stand, there must not be picking and choosing among possible combinations. Appellant respectfully submits that in the appealed rejections, the Office has improperly picked and chosen from among possible combinations, to arrive at the claimed invention. Appellant submits that the rejection improperly utilizes Appellant's disclosure as a guide to pick and choose from Nakamura's broad disclosure, in an attempt to arrive at Appellant's claimed subject matter.

In the Office Action mailed August 18, 2005, the Examiner states that "Nakamura teaches a lyophilized preparation comprising the following components: 1 mg HGF, 100 ml of phosphate buffer, 0.15 M NaCl (see column 14, lines 25-35)." (Office Action mailed August 18, 2005, page 4, lines 5-7.) The Examiner's reliance on this particular disclosure of Nakamura is noteworthy because the Examiner asserts that it provides: a lyophilized preparation of HGF, sodium chloride, and a buffering agent, which is prepared from an aqueous solution containing the hepatocyte growth factor at a concentration lower than 5 mg/mL. The Examiner admits that this composition, i.e., the composition of Example 1 of Nakamura, does not include arginine. (Advisory Action, page 2, lines 14-15.)

In the final Office Action, the Examiner asserts that Appellant's claimed stabilizer, arginine, is disclosed in Nakamura, referring to column 9, lines 52-58. Thus, the Examiner asserts that Nakamura discloses all of the elements of Appellant's claimed invention.

So that there is no question about what Nakamura actually discloses, the passage at column 14, lines 25-35 is reproduced as follows:

Example 1

An aqueous solution is prepared aseptically by adding 1 mg of a hepatocyte growth factor and 100 mg of human serum albumin to 100 ml of 0.02 M phosphate buffer (pH 7.4) containing 0.15 M NaCl and 0.01% Polysorbate 80, and filled in a vial at 1 ml per vial, followed by lyophilization and sealing. Injectable distilled water is filled in an ampoule at 1 ml each for dissolution.

(Nakamura, column 14, lines 25-35.) The passage the Examiner relies upon for the disclosure of arginine is set forth in its entirety, as follows:

The therapeutic agents for hepatocirrhosis of the invention may contain other additives such as stabilizers, excipients, dissolution-promoters, adsorption-preventors and antioxidants, and examples thereof include, for example, sugars such as mannitol and glucose, amino acids such as glycine, alanine, lysine and arginine, proteins such as albumin, alcohols such as ethylene glycol and glycerol, hydrophilic polymers such as polyethylene glycol, inorganic salts such as NaCl, organic salts such as sodium citrate, surfactants such as Polysorbate 80 and reducing agents containing sulfur, which may be used alone or in combination.

(Nakamura, column 9, line 52 – column 10, line 6.)

Appellant respectfully disagrees with the statements that arginine is described in Nakamura as a stabilizing agent. Arginine, along with glycine, alanine, and lysine, are described as “amino acids,” but are not characterized by Nakamura as being anything other than “additives.” To be precise, Nakamura describes additives as including stabilizers, excipients, dissolution-promoters, adsorption-preventors, and antioxidants. Nakamura further discloses “examples” of additives as including, for example, sugars, amino acids, proteins, alcohols, hydrophilic polymers, inorganic salts, organic salts, surfactants, and reducing agents. However, Nakamura does not state how the examples correlate with the classes of additives that are listed. Thus, while Appellant’s specification states that arginine, as well as other amino acids, can be used as stabilizers, that information is not provided by Nakamura, and to suggest that Nakamura discloses arginine – or any other particular amino acid – for use as a stabilizer, is factually incorrect.

Concerning this particular point, the Advisory Action states that “the Examiner notes that Nakamura lists arginine in the same sentence as ‘stabilizers.’ It appears that Nakamura

contemplated that arginine had stabilizing properties.” (Advisory Action, continuation sheet, lines 40-41.) The 87-word “sentence” referred to by the Examiner is set forth above in its entirety. Appellant respectfully submits that the referred-to passage no more suggests that arginine is a stabilizer than it does that arginine is an excipient, a dissolution-promoter, an adsorption-preventor, and an antioxidant.

Appellant notes that Example 1 from Nakamura (column 14, lines 25-35) contains, in addition to the HGF, phosphate buffer, and NaCl, human serum albumin and Polysorbate 80. Example 1 does not contain arginine. The Example does not provide an indication as to the intended functions of the various components, does not include any amino acid, let alone arginine. Nakamura specifically included certain “additives” in Example 1 – human serum albumin and Polysorbate 80 – but it did not include arginine. And there is no teaching or suggestion in Nakamura as to any desirability to add any other ingredient in the Example 1 composition.

For the foregoing reasons, Appellant respectfully submits that claims 1 and 3 are not anticipated, and respectfully requests withdrawal of the rejection for anticipation over Nakamura.

2. Rejection of Claim 4

The rejection of claim 4 under 35 U.S.C. § 102(b) as being anticipated by Nakamura is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 4 is dependent upon and includes the subject matter recited in claim 1. Therefore, the anticipation rejection based upon claim 4 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

3. Rejection of Claim 6

The rejection of claim 6 under 35 U.S.C. § 102(b) as being anticipated by Nakamura is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 6 is dependent upon and includes the subject matter recited in claim 1. Therefore, the anticipation rejection based upon claim 6 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

4. Rejection of Claim 7

The rejection of claim 7 under 35 U.S.C. § 102(b) as being anticipated by Nakamura is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 7 is dependent upon and includes the subject matter recited in claim 1. Therefore, the anticipation rejection based upon claim 7 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura does not teach the combination of features as recited in claim 7, which further includes that the buffering agent is a phosphoric acid salt.

Accordingly, the anticipation rejection based upon Nakamura should be withdrawn.

5. Rejection of Claim 8

The rejection of claim 8 under 35 U.S.C. § 102(b) as being anticipated by Nakamura is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 8 is dependent upon and includes the subject matter recited in claim 1. Therefore, the anticipation rejection based upon claim 8 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura does not teach the combination of features as recited in claim 8, which further requires that the aqueous solution before lyophilization have a pH and an osmotic pressure ratio desirable as an injection.

Accordingly, the anticipation rejection based upon Nakamura should be withdrawn.

6. Rejection of Claim 9

The rejection of claim 9 under 35 U.S.C. § 102(b) as being anticipated by Nakamura is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 9 is dependent upon and includes the subject matter recited in claim 1. Therefore, the anticipation rejection based upon claim 9 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura does not teach the combination of features as recited in claim 9, which further requires that the aqueous solution obtained after redissolution have a pH and an osmotic pressure ratio desirable as an injection.

Accordingly, the anticipation rejection based upon Nakamura should be withdrawn.

7. Rejection of Claim 12

The rejection of claim 12 under 35 U.S.C. § 102(b) as being anticipated by Nakamura is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 12 is dependent upon and includes the subject matter recited in claim 1. Therefore, the anticipation rejection based upon claim 12 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura does not teach the combination of features as recited in claim 12, which further requires that the preparation contain a surface active agent.

Accordingly, the anticipation rejection based upon Nakamura should be withdrawn.

8. Rejection of Claim 13

The rejection of claim 13 under 35 U.S.C. § 102(b) as being anticipated by Nakamura is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 13 is dependent upon and includes the subject matter recited in claim 12. Therefore, the anticipation rejection based upon claim 13 is without appropriate basis for at least the reasons set forth by Appellant with respect to claims 12 and 1.

Moreover, Nakamura does not teach the combination of features as recited in claim 13, which further requires that the surface active agent be a nonionic surface active agent.

Accordingly, the anticipation rejection based upon Nakamura should be withdrawn.

9. Rejection of Claim 14

The rejection of claim 14 under 35 U.S.C. § 102(b) as being anticipated by Nakamura is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 14 is dependent upon and includes the subject matter recited in claim 13. Therefore, the anticipation rejection based upon claim 14 is without appropriate basis for at least the reasons set forth by Appellant with respect to claims 13, 12, and 1.

Moreover, Nakamura does not teach the combination of features as recited in claim 14, which further requires that the nonionic surface active agent be a polyoxyethylene ether surface active agent.

Accordingly, the anticipation rejection based upon Nakamura should be withdrawn.

10. Rejection of Claim 15

The rejection of claim 15 under 35 U.S.C. § 102(b) as being anticipated by Nakamura is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 15 is dependent upon and includes the subject matter recited in claim 1. Therefore, the anticipation rejection based upon claim 15 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura does not teach the combination of features as recited in claim 15, which further requires that the preparation be prepared in a vial or ampoule.

Accordingly, the anticipation rejection based upon Nakamura should be withdrawn.

11. Rejection of Claim 16

The rejection of claim 16 under 35 U.S.C. § 102(b) as being anticipated by Nakamura is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 16 is dependent upon and includes the subject matter recited in claim 1. Therefore, the anticipation rejection based upon claim 16 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura does not teach the combination of features as recited in claim 16, which further requires the stabilizing agent in an amount sufficient to prevent HGF aggregate formation during at least one of lyophilization and storage after the lyophilization.

Accordingly, the anticipation rejection based upon Nakamura should be withdrawn.

B) Whether claims 1 and 16 are anticipated under 35 U.S.C. § 102(b) by, or in the alternative, obvious under 35 U.S.C. § 103(a) over, Nakamura.

The Office Action mailed August 18, 2005 sets forth the details of this rejection, noting:

The reasons why the teachings of Nakamura meet the limitations of claim 1 are presented in the previous paragraphs. Claim 16 is drawn to an amount of the stabilizing agent sufficient to prevent HGF aggregate formation during lyophilization and/or storage after lyophilization. The Examiner cannot determine if the amount used by Nakamura is sufficient to achieve the claimed property, prevention of aggregate formation.

(Office Action mailed August 18, 2005, page 4, line 31 – page 5, line 3.)

Appellant notes that while the Examiner states that claim 1 is anticipated by, or in the alternative, obvious over, Nakamura, the Examiner fails to make any case for the obviousness of this claim. The Examiner fails to address any of the three requirements of a *prima facie* case of obviousness: motivation, expectation of success, and presence of all claimed elements. For this technical deficiency alone, the rejection should be withdrawn.

However, Appellant additionally notes that a *prima facie* case cannot be made from Nakamura for at least the following reasons. As noted above, the specific Examples disclosed by Nakamura do not include arginine. Moreover, there is nothing in Nakamura that would cause one of ordinary skill in the art to add arginine, or to replace another component of Nakamura's Examples with arginine. There is no suggestion of its desirability as an additional additive, and thus, there is no reason one of skill in the art would add it to Nakamura's exemplified compositions. Additionally, there is no suggestion of its interchangeability with some other component already present in one of Nakamura's compositions. For these reasons, there is no reason that a person of skill in the art would select, from all of the choices of "additives" in

Nakamura, arginine. There is no teaching or suggestion in Nakamura to arrive at Appellant's claim.

Additionally, there is not a reasonable expectation of success in a modification of Nakamura that would result in the presently claimed invention. Appellant respectfully refers the Board to the "Background Art" section of the present specification, which describes at least two published HGF formulations, examples of which are described as including, for example, human serum albumin, mannitol, lysine, arginine, glycine, and alanine, as stabilizing agents. However, each of these formulations is described as being unacceptable: one for lack of long-term stability, and the other for being undesirable for human administration. It is respectfully submitted that modifying or changing the additives in HGF formulations can result in unexpected results and undesirable final products. Without more, there is no reasonable expectation of success in a modification of Nakamura.

It is unclear why claim 1 is included in this rejection, as it does not include the "amount . . . sufficient" element of claim 16, which is apparently the point of this rejection. Additionally, the rejection makes clear that the Examiner believes that all of the elements of claim 1 are explicitly taught by Nakamura ("The reasons why the teachings of Nakamura meet the limitations of claim 1 are presented in the previous paragraphs.") That this rejection is intended for claim 16 is reinforced by the Advisory Action, which states that "Rejections under §§ 102/103 are appropriate when the prior art reference appears to disclose the claimed invention except that it is silent as to an inherent property. MPEP 2112(III). Here, the reference is silent as to whether or not the amount of arginine included is sufficient to prevent HGF aggregate formation." (Advisory Action, page 3, lines 11-13.)

Appellant respectfully notes that Nakamura has been discussed above in detail, as to the rejection of claim 1 for anticipation. Appellant notes that claim 16 depends from claim 1 and thus, includes all of the same elements as claim 1. To the extent that Appellant's points above were made with regard to the elements recited in claim 1, they are equally applicable to claim 16. Additionally, Appellant respectfully submits that there is nothing in Nakamura that suggests that the amount of stabilizing agent should be sufficient to prevent HGF aggregate formation. In this

regard, Appellant notes that Nakamura makes no mention of any stability problem, either with or without lyophilization. Thus, Appellant respectfully submits that there is nothing in Nakamura that would suggest the use of an amount of a stabilizing agent sufficient to prevent aggregation.

For at least these reasons, Appellant respectfully submits that Nakamura does not anticipate or render obvious claim 1 or 16, and respectfully requests withdrawal of the rejections for anticipation or obviousness over Nakamura.

C) Whether claims 1, 3, 4, and 6-16 are obvious under 35 U.S.C. § 103(a) over Nakamura (European Application No. 0456188 A1) in view of Tanaka (WO 97/02832).

1. Rejections of Claims 1 and 3

Initially, with regard to Tanaka, Appellant notes that the Office Action indicates that the rejection is over Nakamura (European Application No. 0456188 A1) in view of Tanaka (WO 97/02832), “as evidenced by Tanaka et al. (U.S. Patent Application Publication 2001/0051604, published 13 December 2001, cited by Applicant on IDS filed 27 February 2004).” Thus, the Examiner’s specific rejection and Appellant’s response thereto relate to the disclosure of U.S. Patent Application Publication 2001/0051604.

Appellant has noted above the reasons why Nakamura does not anticipate or render obvious the presently claimed invention. Still further, Appellant respectfully submits that Tanaka et al. fails to supply Nakamura’s missing teachings and also fails to provide motivation to make any change to Nakamura to arrive at the presently claimed invention.

The Office Action admits that Nakamura does not disclose Appellant’s specifically claimed pH range (see claim 10, for example), but relies upon Tanaka et al. for this missing teaching. However, Appellant respectfully submits that a *prima facie* case of obviousness does not result. Initially, Appellant notes that there is nothing in Nakamura that would lead to the selection of a different pH than that disclosed, i.e., pH 7.4. While it is not explicitly stated, it is reasonable to conclude that the choice of pH 7.4 was made to closely match physiological pH.

However, there is nothing in Nakamura that would suggest that such pH is undesirable. Thus, there is no reason to turn to the disclosure of Tanaka et al. for the choice of a different pH.

Moreover, if anything, Tanaka et al. teaches away from the present invention, which requires a concentration of less than 5 mg/ml. Tanaka et al. specifically states that the solubility of HGF varies with pH and that the solubility is 0.1 to 5 mg/ml at pH 7, but the solubility is over 20 mg/ml at pH 5. (Tanaka et al., paragraph [0018]). Tanaka et al. then proceeds to state that therefore, “it is *preferred* to keep the pH around 5.0 to 6.0.” (Id., emphasis added.) Thus, Tanaka et al. clearly suggests a higher concentration of HGF than 5 mg/ml.

Appellant respectfully submits that a *prima facie* case of obviousness of claims 1 and 3 does not result from the combination of Nakamura and Tanaka and respectfully requests withdrawal of the rejection for obviousness.

2. Rejection of Claim 4

The rejection of claim 4 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 4 is dependent upon and includes the subject matter recited in claim 1. Therefore, the obviousness rejection of claim 4 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

3. Rejection of Claim 6

The rejection of claim 6 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 6 is dependent upon and includes the subject matter recited in claim 1. Therefore, the obviousness rejection of claim 6 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

4. Rejection of Claim 7

The rejection of claim 7 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 7 is dependent upon and includes the subject matter recited in claim 1. Therefore, the obviousness rejection of claim 7 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura in view of Tanaka do not suggest the features as recited in claim 7, which further includes that the buffering agent is a phosphoric acid salt.

Accordingly, the obviousness rejection based upon Nakamura in view of Tanaka should be withdrawn.

5. Rejection of Claim 8

The rejection of claim 8 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 8 is dependent upon and includes the subject matter recited in claim 1. Therefore, the obviousness rejection of claim 8 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura in view of Tanaka do not teach the combination of features as recited in claim 8, which further requires that the aqueous solution before lyophilization have a pH and an osmotic pressure ratio desirable as an injection.

Accordingly, the obviousness rejection based upon Nakamura in view of Tanaka should be withdrawn.

6. Rejection of Claim 9

The rejection of claim 9 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 9 is dependent upon and includes the subject matter recited in claim 1. Therefore, the obviousness rejection based upon claim 9 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura in view of Tanaka do not teach the combination of features as recited in claim 9, which further requires that the aqueous solution obtained after redissolution have a pH and an osmotic pressure ratio desirable as an injection.

Accordingly, the obviousness rejection based upon Nakamura in view of Tanaka should be withdrawn.

7. Rejection of Claim 10

The rejection of claim 10 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 10 is dependent upon and includes the subject matter recited in claim 1. Therefore, the obviousness rejection based upon claim 10 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura in view of Tanaka do not teach the combination of features as recited in claim 10, which further requires that a pH of the aqueous solution before lyophilization be in the range of 5 to 6.5.

Accordingly, the obviousness rejection based upon Nakamura in view of Tanaka should be withdrawn.

8. Rejection of Claim 11

The rejection of claim 11 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 11 is dependent upon and includes the subject matter recited in claim 1. Therefore, the obviousness rejection based upon claim 11 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura in view of Tanaka do not teach the combination of features as recited in claim 11, which further requires that a pH of the aqueous solution obtained after redissolution be in the range of 5 to 6.5.

Accordingly, the obviousness rejection based upon Nakamura in view of Tanaka should be withdrawn.

9. Rejection of Claim 12

The rejection of claim 12 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 12 is dependent upon and includes the subject matter recited in claim 1. Therefore, the obviousness rejection based upon claim 12 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura in view of Tanaka do not teach the combination of features as recited in claim 12, which further requires that the preparation contain a surface active agent.

Accordingly, the obviousness rejection based upon Nakamura in view of Tanaka should be withdrawn.

10. Rejection of Claim 13

The rejection of claim 13 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 13 is dependent upon and includes the subject matter recited in claim 12. Therefore, the obviousness rejection of claim 13 is without appropriate basis for at least the reasons set forth by Appellant with respect to claims 12 and 1.

Moreover, Nakamura in view of Tanaka do not teach the combination of features as recited in claim 13, which further requires that the surface active agent be a nonionic surface active agent.

Accordingly, the obviousness rejection based upon Nakamura in view of Tanaka should be withdrawn.

11. Rejection of Claim 14

The rejection of claim 14 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 14 is dependent upon and includes the subject matter recited in claim 13. Therefore, the obviousness rejection of claim 14 is without appropriate basis for at least the reasons set forth by Appellant with respect to claims 13, 12, and 1.

Moreover, Nakamura in view of Tanaka do not teach the combination of features as recited in claim 14, which further requires that the nonionic surface active agent be a polyoxyethylene ether surface active agent.

Accordingly, the obviousness rejection based upon Nakamura in view of Tanaka should be withdrawn.

12. Rejection of Claim 15

The rejection of claim 15 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 15 is dependent upon and includes the subject matter recited in claim 1. Therefore, the obviousness rejection based upon claim 15 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura in view of Tanaka do not teach the combination of features as recited in claim 15, which further requires that the preparation be prepared in a vial or ampoule.

Accordingly, the obviousness rejection based upon Nakamura in view of Tanaka should be withdrawn.

13. Rejection of Claim 16

The rejection of claim 16 under 35 U.S.C. § 103(a) as obvious over Nakamura in view of Tanaka is in error, the decision of the Examiner to finally reject this claim should be reversed, and the application should be remanded to the Examiner.

Appellant notes that claim 16 is dependent upon and includes the subject matter recited in claim 1. Therefore, the obviousness rejection based upon claim 16 is without appropriate basis for at least the reasons set forth by Appellant with respect to claim 1.

Moreover, Nakamura in view of Tanaka do not teach the combination of features as recited in claim 16, which further requires the stabilizing agent in an amount sufficient to prevent HGF aggregate formation during at least one of lyophilization and storage after the lyophilization.

Accordingly, the obviousness rejection based upon Nakamura in view of Tanaka should be withdrawn.

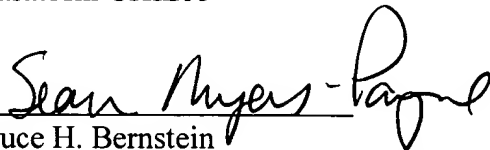
CONCLUSION

Each of claims 1, 3, 4, and 6-16 is patentable for the reasons set forth herein. Specifically, the applied art of record does not teach or suggest the combination of features recited in Appellant's claims, and is not combinable in the manner proposed by the Examiner, and even if it were considered to be properly combined, fails to disclose or suggest the unique combination of features recited in Appellant's claims 1, 3, 4, and 6-16. Appellants respectfully request that the Board reverse the decision of the Examiner to reject claims 1, 3, 4, and 6-16, and remand the application to the Examiner for withdrawal of the rejection.

Application No. 09/926,661
Attorney Docket No. P21749
Amended Appeal Brief Under 37 C.F.R. § 41.37(d)

Thus, Appellants respectfully submit that each and every pending claim of the present application meets requirements for patentability, and that the present application and each pending claim are allowable over the prior art of record.

Respectfully submitted,
Masatoshi CHIBA



Bruce H. Bernstein
Reg. No. 29,027
42,920

February 7, 2007
GREENBLUM & BERNSTEIN, P.L.C.
1950 Roland Clarke Place
Reston, VA 20191
(703) 716-1191

VIII. Claims Appendix

1. A lyophilized preparation comprising a hepatocyte growth factor, a stabilizing agent comprising arginine, lysine, histidine, glutamine, proline, glutamic acid, or aspartic acid, or a pharmacologically acceptable salt thereof, for preventing formation of an aggregate of the hepatocyte growth factor, sodium chloride, and a buffering agent, which is prepared from an aqueous solution containing the hepatocyte growth factor at a concentration lower than 5 mg/mL.
3. A lyophilized preparation comprising a hepatocyte growth factor, a stabilizing agent comprising arginine, lysine, histidine, glutamine, proline, glutamic acid, or aspartic acid, or a pharmacologically acceptable salt thereof, for preventing formation of an aggregate of the hepatocyte growth factor, sodium chloride, and a buffering agent, which is prepared from an aqueous solution containing the hepatocyte growth factor at a concentration lower than 5 mg/mL, and capable of preparing an aqueous solution containing the hepatocyte growth factor at a concentration lower than 5 mg/mL by redissolution.
4. The lyophilized preparation according to claim 1, wherein the stabilizing agent comprises arginine, lysine, histidine, glutamic acid, or aspartic acid, or a pharmacologically acceptable salt thereof.
6. The lyophilized preparation according to claim 1, wherein the stabilizing agent comprises arginine, lysine, or histidine, or a pharmacologically acceptable salt thereof.
7. The lyophilized preparation according to claim 1, wherein the buffering agent is a phosphoric acid salt.
8. The lyophilized preparation according to claim 1, wherein the aqueous solution before lyophilization has a pH and an osmotic pressure ratio desirable as an injection.
9. The lyophilized preparation according to claim 1, wherein the aqueous solution obtained after redissolution has a pH and an osmotic pressure ratio desirable as an injection.
10. The lyophilized preparation according to claim 1, wherein a pH of the aqueous solution before lyophilization is in the range of 5 to 6.5.
11. The lyophilized preparation according to claim 1, wherein a pH of the aqueous solution obtained after redissolution is in the range of 5 to 6.5.
12. The lyophilized preparation according to claim 1, which further contains a surface active agent.

13. The lyophilized preparation according to claim 12, wherein the surface active agent is a nonionic surface active agent.
14. The lyophilized preparation according to claim 13, wherein the nonionic surface active agent is a polyoxyethylene ether surface active agent.
15. The lyophilized preparation according to claim 1, which is prepared in a vial or an ampoule.
16. The lyophilized preparation according to claim 1, which contains the stabilizing agent in an amount sufficient to prevent HGF aggregate formation during at least one of lyophilization and storage after the lyophilization.

IX. Evidence Appendix

European Application No. 0456188 A1, listed on an Examiner-initialed Form PTO-1449 attached to the Office Action mailed August 18, 2005;

WO 97/02832, listed on a Form PTO-892 attached to the Office Action mailed August 18, 2005;

U.S. Patent Application Publication 2001/0051604, listed on an Examiner-initialed Form PTO-1449 attached to the Office Action mailed August 18, 2005;

Ex parte Bobsein et al., (Appeal No. 2005-1332), cited by Appellant in the Amendment under 37 C.F.R. § 1.116, filed May 16, 2006, at page 9, line 6, and indicated as “fully considered” by the Examiner in the Advisory Action mailed June 8, 2006, at page 2, lines 10-11; and

In re Arkley, 172 U.S.P.Q. 524 (CCPA 1972), cited by Appellant in the Amendment under 37 C.F.R. § 1.116, filed May 16, 2006, at page 9, line 7, and indicated as “fully considered” by the Examiner in the Advisory Action mailed June 8, 2006, at page 2, lines 10-11.

Application No. 09/926,661

Attorney Docket No. P21749

Amended Appeal Brief Under 37 C.F.R. § 41.37(d)

VIII. Related Proceedings Appendix

None.



The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte BARRETT RICHARD BOBSEIN,
WILLIAM CHRISTOPHER FINCH,
and
DAVID ALBERT GLEESON

Appeal No. 2005-1332
Application No. 09/774,064

ON BRIEF

Before PAK, WARREN, and TIMM, *Administrative Patent Judges*.
TIMM, *Administrative Patent Judge*.

DECISION ON APPEAL

This appeal involves claims 1 and 3 which are all the claims pending in the application.
We have jurisdiction over the appeal pursuant to 35 U.S.C. § 134.



INTRODUCTION

Claims 1 and 3 stand rejected under 35 U.S.C. § 102(b) as anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as obvious over Japanese Published Unexamined Application 05-170,802 to Hoshino et al. published on July 9, 1993 (Hoshino).¹

The claims stand or fall together (Brief, p. 4). We select claim 1 to represent the issues on appeal. Claim 1 reads as follows:

1. A waterborne pigmented paper or paperboard coating composition comprising pigment comprising 50% to 100%, by weight of said pigment, calcium carbonate and from 1% to 25%, as dry weight by weight of said pigment, of an aqueous polymeric dispersion comprising
 - (c) 95-25% by weight, based on the weight of the solids of said aqueous polymeric dispersion, of a first emulsion polymer having an average particle diameter of 150 to 3000 nanometers and
 - (d) 5-75% by weight, based on the weight of the solids of said aqueous polymeric dispersion, of a second emulsion polymer having an average particle diameter of 40 to 600 nanometerswherein the ratio of said average particle diameter of said first emulsion polymer to said average particle diameter of said second emulsion polymer is from 1.2 to 60,
wherein at least said first emulsion polymer particles, when dry, contain at least one void, and wherein said first emulsion polymer is prepared in the presence of said second emulsion polymer or said second emulsion polymer is prepared in the presence of said first emulsion polymer.

Because the Examiner has established a prima facie case of obviousness, we affirm. Our reasons follow.

¹We rely upon and cite to the English translation made of record on March 14, 2005.

OPINION

Hoshino describes a waterborne pigmented paper or paperboard coating composition including, among other things, a pigment containing inorganic pigments and emulsion particles as plastic pigments (Hoshino, ¶ 0016, ll. 6-10). Hoshino notes that hard emulsion particles have been studied as additives for coating agents for reducing coating weight, improving gloss, whiteness, opacity, etc. (Hoshino, ¶ 0002, ll. 1-4). According to Hoshino, the industrial use of these emulsion particles as replacements for inorganic pigments such as kaolin, calcium carbonate, talc, satin, etc. in the paper coating field is increasing (Hoshino, ¶ 0002, ll. 4-7).

Hoshino describes emulsion particles with a bimodal particle distribution (Hoshino, ¶ 0009-10). The Examiner finds, and Appellants do not dispute, that the Examples of Hoshino show the claimed proportion and diameters of the two emulsion polymer particles required by claim 1 (Answer, p. 3; Brief and Reply Brief in their entirety). Nor is there any dispute that the emulsion polymer particles of Hoshino meet the other requirements of the aqueous polymeric dispersion recited in claim 1 (Answer, p. 3; Brief and Reply Brief in their entirety). Appellants' arguments focus instead on the calcium carbonate concentration recited in the claim. The issue, therefore, is whether Hoshino sufficiently describes including calcium carbonate in the composition in an amount sufficient to anticipate the composition of the claim or whether there is a sufficient reason, suggestion, or motivation to add calcium carbonate in the claimed amount such that there is a prima facie case of obviousness.

Anticipation

We agree with Appellants that Hoshino does not disclose each and every limitation of claim 1 with sufficient specificity such that the claimed composition is anticipated. In order to anticipate, Hoshino must clearly and unequivocally disclose the claimed invention or direct those skilled in the art to the invention without any need for picking, choosing, and combining various disclosures not directly related to each other by the teachings of the cited reference. *In re Arkley*, 455 F.2d 586, 587, 172 USPQ 524, 526 (CCPA 1972). "Such picking and choosing may be entirely proper in the making of a 103, obviousness rejection, where the applicant must be afforded an opportunity to rebut with objective evidence any inference of obviousness which may arise from the similarity of the subject matter which he claims to the prior art, but it has no place in the making of a 102, anticipation rejection." *Arkley*, 455 F.2d at 587-88, 172 USPQ at 526.

The Examiner's finding of anticipation is based upon the disclosure in Hoshino of a concentration of aqueous polymeric dispersion in the range of 3-30% as a preferred embodiment coupled with a disclosure calcium carbonate in a list of six inorganic pigments. But Hoshino, in fact, does not limit the inorganic pigments to the six compounds specifically recited. What Hoshino states is that "[s]ome examples of the inorganic pigments include kaolin, calcium carbonate, talc, satin white, titanium dioxide, etc." Moreover, the only exemplified composition contains an inorganic pigment mixture of 63 parts of kaolin clay with 27 parts of calcium carbonate. Therefore, mixtures are also contemplated. One of ordinary skill in the art, in fact, is

directed to picking and choosing an inorganic pigment from a much larger genus than acknowledged by the Examiner. Moreover, there is no direct disclosure of a pigment mixture containing an amount of calcium carbonate within the claimed range coupled with an amount of emulsion particles in the claimed range of 1-25%. To obtain the composition of claim 1, one of ordinary skill in the art must both pick and choose among the various acceptable inorganic pigments and conduct some experimentation, albeit routine in nature, with regard to the amount of inorganic pigment and emulsion particles to include in the pigment. Therefore, we find the disclosure of Hoshino lacks the specificity required for a finding of anticipation.

Obviousness

The question of obviousness, however, stands on a different footing. As stated above, picking and choosing within the teachings of the prior art is entirely proper in the context of an obviousness rejection. *Arkley*, 455 F.2d at 587-88, 172 USPQ at 526. Routine experimentation involving such parameters as concentration is also proper in the context of obviousness. *See In re Boesch*, 617 F.2d 272, 276, 205 USPQ 215, 219 (CCPA 1980). Note also *In re Woodruff*, 919 F.2d 1575, 1578, 16 USPQ2d 1934, 1936-37 (Fed. Cir. 1990), and *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Claim 1 requires that calcium carbonate be present in the pigment in an amount of 50-100 weight %. The claim further requires that the aqueous dispersion of emulsion polymers be present in an amount of 1-25%, as dry weight by weight of the pigment. The Examiner finds that Hoshino describes, as a preferred embodiment, including the emulsion polymer particles in

an amount of 3-30% by weight of the pigment and concludes, therefore, that the inorganic pigment must be present in an amount of 70-97% by weight of the pigment in that preferred embodiment (Answer, p. 3). Appellants traverse this finding on the basis that “this is not the literal disclosure of Hoshino.” (Brief, p. 4). Appellants’ traversal is not persuasive because, even though Hoshino does not say it literally, the disclosure is present. The pigment of Hoshino is a combination of inorganic pigments and the emulsion particles as “plastic pigment” (Hoshino, ¶ 0016, ll. 6-9). The amount of emulsion particles is related in Hoshino as a percentage of the “entire pigments.” (Hoshino, ¶ 0017, ll. 1-4). Therefore, the percentage of inorganic pigments is the amount which is not emulsion pigment.² We, therefore, find adequate factual support in Hoshino for the finding made by the Examiner, i.e., that Hoshino describes by default including inorganic pigment in an amount of from between 97 and 70% by weight of the entire pigment in the preferred embodiment. That Hoshino includes other less preferred embodiments and examples does not, contrary to the arguments of Appellants (Brief, p. 5), somehow negate the disclosure of the preferred embodiment.

²The words “entire pigments” would be understood by one of ordinary skill in the art to be referring to the combination of emulsion particles as plastic pigments and inorganic pigments. This is the case because inorganic and plastic pigments are the only components that make up the pigment. In fact, the plastic pigments are said to be a replacement for inorganic pigments (Hoshino, ¶ 0002, ll. 4-7). Also note that Hoshino calculates the quantity of other components based on the combined amount of inorganic and plastic pigments (Hoshino, ¶ 0016, ll. 19-22). Moreover, the formulation provided on page 22 of the translation of Hoshino further validates the Examiner’s interpretation of the reference as the pigment amounts (clay, calcium carbonate and emulsion particles) add up to 100 parts by weight.

We agree with the Examiner that it would have been obvious to one of ordinary skill in the art to select calcium carbonate as the inorganic pigment in the composition of Hoshino as it is expressly suggested in the reference. It follows then that Hoshino suggests the use of a pigment containing 70-97% by weight calcium carbonate as required by claim 1.

Appellants argue that the Examiner has not met his burden in establishing a *prima facie* case of obviousness because he has not pointed to any disclosure within Hoshino which indicates a realization of the problem faced by Appellants or which would motivate one skilled in the art to form Appellants' composition (Brief, p. 6). This argument is not persuasive for several reasons. First, the prior art need not address Appellants' problem. *In re Dillon*, 919 F.2d 688, 693, 16 USPQ2d 1897, 1901-1902 (Fed. Cir. 1990)(*en banc*), *cert. denied*, 500 U.S. 904 (1991). Second, Hoshino recognizes both gloss and brightness (whiteness), the properties focused on by Appellants, as important properties to be optimized (Hoshino, ¶ 0008). Third, Hoshino describes dispersions having the bimodal particle composition claimed, describes calcium carbonate as one of the inorganic pigments which can be combined with the emulsion particles and suggests amounts within and/or overlapping those of the claim. Under these circumstances, a case of *prima facie* obviousness is properly established. Where the difference between the claimed invention and the prior art is some range or other variable within the claims, the applicant must show that the particular range is critical, generally by showing that the claimed range achieves unexpected results relative to the prior art range. *In re Woodruff*, 919 F.2d 1575, 1578, 16 USPQ2d 1934, 1936-37 (Fed. Cir. 1990).

We conclude that the Examiner has established a prima facie case of obviousness with respect to the subject matter of claims 1 and 3 which has not been sufficiently rebutted by Appellants. To the extent that Appellants are relying upon a showing of unexpected results to overcome the prima facie case of obviousness, we note that sufficiently probative objective evidence has not been relied upon in this appeal. Attorney arguments in the brief cannot take the place of evidence. *In re Lindner*, 457 F.2d 506, 508, 173 USPQ 356, 358 (CCPA 1972).

CONCLUSION

To summarize, the decision of the Examiner to reject claims 1 and 3 under 35 U.S.C. § 102(b) or, in the alternative, under 35 U.S.C. § 103(a) is affirmed on the basis of obviousness under § 103(a).

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

AFFIRMED

CHUNG K. PAK
Administrative Patent Judge

CHARLES F. WARREN
Administrative Patent Judge

CATHERINE TIMM
Administrative Patent Judge

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CT/jrg

Appeal No. 2005-1332
Application No. 09/774,064

Page 10

Ronald D. Bakule
Rohm and Haas Company
Patent Department
100 Independence Mall West
Philadelphia, PA 9106-2399

[2] Defendant, by its answer, asserts that the patents in question are invalid for 14 different reasons. Misjoinder or nonjoinder of inventors is simply one of the reasons defendant has alleged. This Court can see no advantage in granting a separate hearing on the issue of nonjoinder or misjoinder; indeed, if such a hearing were granted, the parties might have to produce the same witnesses and evidence two different times. This Court is of the opinion that the issue of nonjoinder or misjoinder of inventors is no more of a threshold legal issue than any of the other grounds asserted for patent invalidity. Accordingly, this Court holds defendant has no right to a separate hearing on the issue of patent invalidity due to misjoinder or nonjoinder of inventors under Rule 42(b) of the Federal Rules of Civil Procedure.

III. Rule 12(d)

Rule 12(b) (7) of the Federal Rules of Civil Procedure allows a party to move to dismiss a claim for failure to join a party under Rule 19. Rule 12(d) states:

The defenses specifically enumerated (1)-(7) in subdivision (b) of this rule, whether made in a pleading or by motion * * * shall be heard and determined before trial on application of any party, unless the court orders that the hearing and determination thereof be deferred until the trial.

[3] Defendant apparently asserts the alleged nonjoined or misjoined inventors are necessary parties to this suit under Rule 19. This contention is without merit. The inventors are not necessary parties for a just adjudication of this suit; they are only involved tangentially in the instant case in that their nonjoinder or misjoinder in the patent application may have rendered the patent invalid. Accordingly, this Court holds defendant has no right to a separate hearing on the issue of patent invalidity due to misjoinder or nonjoinder of inventors under Rule 12(d) of the Federal Rules of Civil Procedure.

Accordingly, it is hereby ordered, adjudged and decreed that defendant's motion for a separate hearing on the issue of patent invalidity due to nonjoinder or misjoinder of inventors is denied.

Court of Customs and Patent Appeals

In re ARKLEY, EARDLEY, AND LONG

No. 8553

Decided Feb. 17, 1972

PATENTS

1. Patentability — Anticipation — In general (§51.201)

Patentability — Invention — In general (§51.501)

Fact that rejections under 35 U.S.C. 103 are proper where subject matter claimed "is not identically disclosed or described" in prior art indicates that rejections under section 102 are proper only when claimed subject matter is identically disclosed or described in prior art.

2. Court of Customs and Patent Appeals — In general (§28.01)

Court does not grant patent where it reverses rejection of claim; it is Patent Office which grants patents, not the court.

3. Court of Customs and Patent Appeals — In general (§28.01)

Pleading and practice in Patent Office — Rejections (§54.7)

Court's reversal of rejection of claim on ground that it is anticipated by reference under 35 U.S.C. 102 leaves Patent Office free to reject claim as obvious under section 103 in view of reference since such latter rejection was not before court.

4. Court of Customs and Patent Appeals — Weight given decisions below (§28.35)

It is not court's practice to apply a different standard in cases in complex areas of technology than it does in easily understood cases.

Particular patents—Cephaloridine

Arkley, Eardley, and Long, Cephaloridine, rejection of claim 30 reversed.

Appeal from Board of Appeals of the Patent Office.

Application for patent of Vincent Arkley, Stephen Eardley, and Alan Gibson Long, Serial No. 329,212, filed Dec. 9, 1963; Patent Office Group 120. From decision rejecting claim 30, applicants appeal. Reversed; Baldwin, Judge, concurring with opinion in which

Almond, Judge, joins; Worley, Chief Judge, dissenting with opinion.

J. WILLIAM PIKE and BACON & THOMAS, both of Washington, D. C. (FRED T. WILLIAMS, JOHN J. CAVANAUGH, and PENDLETON, NEUMAN, WILLIAMS & ANDERSON of counsel) for appellants.

S. WM. COCHRAN (JACK E. ARMORE and HENRY WILLARD TARRING II of counsel) for Commissioner of Patents.

Before WORLEY, Chief Judge, and RICH, ALMOND, BALDWIN, and LANE, Associate Judges.

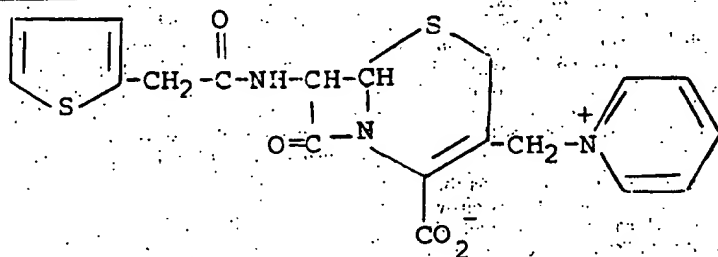
RICH, Judge:

This appeal is from the decision of the Patent Office Board of Appeals affirming the rejection of claim 30 in appellants' application serial No. 329,212, filed December 9, 1963, for a cephalosporin-type antibiotic known as cephaloridine. No claim has been allowed. We reverse.

The Subject Matter Claimed

The appealed claim is drawn to a single compound, by structural formula, and reads:

30. A compound of the formula

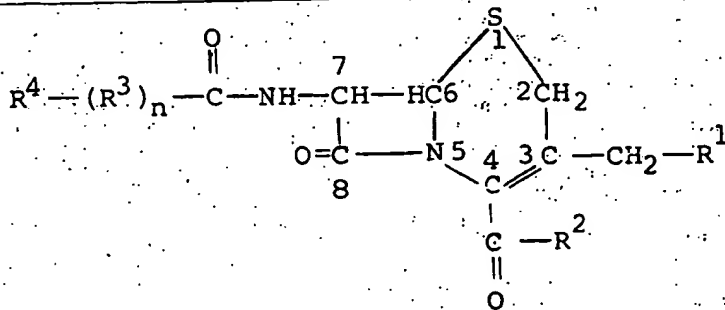


This compound is said to be a broad spectrum antibiotic, effective against both gram-positive and gram-negative micro-organisms, and to possess many other virtues not relevant here because of the nature of the rejection.

The Rejection

Appellants' claim has been rejected as anticipated by U. S. patent No. 3,218,318,

issued to Edwin H. Flynn November 16, 1965, on an application filed in the United States August 31, 1962, and available against appellants' application by virtue of 35 U.S.C. 102(e) as of its filing date. This reference discloses generically a class of cephalosporin-type compounds having the following structural formula:



in which R^1 , taken alone, is $-\text{OH}$, $\text{C}_1\text{-Cs}$ acyloxy, or tertiary-amino, R^2 is $-\text{OH}$ when R^1 is $-\text{OH}$, R^2 is $-\text{OH}$ when R^1 is $\text{C}_1\text{-Cs}$ acyloxy, R^2 is $-\text{O}-$ when R^1 is tertiary-amino, R^1 and R^2 , when taken together, are $-\text{O}-$, n is zero or 1, R^3 is $\text{C}_1\text{-Cs}$ alkylene, and R^4 is a heteromonocyclic radical containing O , S , and/or N . Appellants "conservatively" estimate that over 230,000 compounds (including, concededly, theirs) are embraced within this generic disclosure, and

the board in turn conceded that, "If this were the only anticipatory disclosure in the reference," the disclosure would be "too diffuse" to support a 102 rejection.

However, the board found: (1) that Flynn's examples 4 and 10 "adequately disclose the exact precursors of the presently claimed compound"; (2) that Flynn's statement that

Cephalosporin C is also readily converted into compounds of the cephalosporin C_A

type by refluxing in aqueous solution with an excess of pyridine, for example, as described in Belgian Patent 593,777.

was adequate to teach how to convert the C-type precursors disclosed in examples 4 and 10 to the C_A-type compound claimed by appellants; and (3) that Flynn's statement that, "in general, those compounds which possess the cephalosporin C_A nucleus are more effective antibacterially than those containing the cephalosporin C nucleus" provided the "motive . . . to follow this additional teaching . . ." Putting these three findings together, the board held that

The indicated combination of Example 4 or 10 with . . . [the teaching of how to convert "Cephalosporin C . . ." into compounds of the cephalosporin C_A type"] is not a matter of obviousness within the meaning of 35 U.S.C. 103 but of direct teaching within the four corners of the patent.

The effect of this holding, of course, was that the board did not have to look at the extensive objective evidence which appellants had offered to rebut any inference of obviousness which might be thought to arise from the teachings of the Flynn patent.

Opinion

[1] The sole issue in this case is whether cephaloridine is "described" in the Flynn patent within the meaning of that word in 35 U.S.C. 102(e). It is to be noted that rejections under 35 U.S.C. 103 are proper where the subject matter claimed "is not *identically* disclosed or described" (emphasis ours) in "the prior art," indicating that rejections under 35 U.S.C. 102 are proper only when the claimed subject matter is *identically* disclosed or described in "the prior art." Thus, for the instant rejection under 35 U.S.C. 102(e) to have been proper, the Flynn reference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without any need for picking, choosing, and combining various disclosures not directly related to each other by the teachings of the cited reference. Such picking and choosing may be entirely proper in the making of a 103, obviousness rejection, where the applicant must be afforded an opportunity to rebut with objective evidence any inference of obviousness which may arise from the similarity of the subject matter which he claims to

At one time appellants contended that Flynn was not an "enabling disclosure." In re LeGrice, 49 CCPA 1124, 301 F.2d 929, 133 USPO 365 (1962), but we gather that they have abandoned that contention on appeal, although there is still an ambiguous reference to LeGrice in their briefs.

the prior art; but it has no place in the making of a 102, anticipation rejection.

In this case we have no difficulty in deciding that the portions of the Flynn reference relied upon by the Patent Office do not *identically* describe the claimed subject matter. As appellants point out, the compounds of Flynn's examples 4 and 10 are the "exact precursors" of appellants' compound "only to the extent that appellants have discovered that cephaloridine will be formed if the acid [disclosed in example 10] is first selected and then carefully reacted with a particular tertiary amine *which also must be selected*." (Emphasis in original.) Of course, it does appear that the "particular tertiary amine" to which appellants refer is pyridine, which is mentioned elsewhere in Flynn as an example of the class of reactants² with which a particular cephalosporin C-type compound (namely, cephalosporin C itself) may be converted into compounds of the cephalosporin C_A type, but there is nothing in the teachings relied upon by the Patent Office which "clearly and unequivocally" directs those skilled in the art to make this selection nor any indication that Flynn ever made the selection himself. Similarly, while it is reasonable to suppose that Flynn's teaching that "in general, those compounds which possess the cephalosporin C_A nucleus are more effective antibacterially than those

² The parties argue, in essence, about whether the words "for example" in the sentence "Cephalosporin C is also readily converted into compounds of the cephalosporin C_A type by refluxing in aqueous solution with an excess of pyridine, for example, as described in Belgian Patent 593,777" refers to the word "pyridine" or the words "as described." Appellants argue that "it is to be stressed that pyridine is only being suggested as an *example* of the tertiary amine(s) suitable for the reaction with the prior art compound cephalosporin C," while the solicitor seems to be taking the position that Flynn's specification would be read as indicating that the Belgian patent was one place among many where those skilled in the art could learn how to react cephalosporin C with pyridine. While the matter is not free from doubt, we think it more likely that the sentence would be read in the former way because the presence of the word "type" after "C_A" and not after "C" suggests that one particular C-type compound (namely, cephalosporin C itself) can be changed into *various* C_A-type compounds by refluxing it with an excess of the proper reactant. This interpretation of the controverted sentence is reinforced by the next sentence in Flynn's specification, which is as follows:

The reaction is applicable in general to the tertiary amines, of which numerous examples are given above, yielding corresponding derivatives of the cephalosporin C_A type wherein the tertiary amine is attached to the methyl group in the 3 position of the thiazine ring, and forms an inner salt with the carboxyl group in the 4 position.

containing the cephalosporin G nucleus" would provide some "motive" for those that followed him to concentrate their investigations on compounds possessing the cephalosporin C_A nucleus, that motivation is a very general one, pointing to no particular one of the myriads of compounds, actual and potential, containing the cephalosporin C_A nucleus.

The board, apparently recognizing the weakness of its position in attempting to arrive at an anticipation by combining the disclosures in examples 4 and 10 with the above-quoted teaching elsewhere in the patent of how to convert a particular, different cephalosporin C-type compound into cephalosporin C_A-type compounds, postulates certain teachings which might have been in the reference patent any one of which, according to it, if present would have removed all doubt concerning the completeness of the anticipation.³ The simple answer to the board's argument is that these teachings were not contained in the Flynn patent and that we do not regard the teachings which were there and which were relied upon below as the equivalent of those which were postulated by the board. We do not read into references things that are not there.

Although the board declined to discuss four relatively recent decisions by this court in cases involving description requirements in various sections of the patent statute⁴ on the ground that "the issue [of anticipation] is essentially a factual one," it did consider the older case of *In re Armstrong*, 47 CCPA 1084, 280 F.2d 132, 126 USPQ 281 (1960), to be "apposite

³ These postulations were contained in the following passage from the board's opinion:

There would be no doubt of the completeness of the anticipation if, paraphrasing column 3, lines 47 to 50, the following language were present at the end of each of Examples 4 and 10:

"This compound is also readily converted into a compound of the cephalosporin C_A type by refluxing in aqueous solution with an excess of pyridine, for example, as described in Belgian Patent 593,777."

Likewise, there would be no question of the applicability of column 3, lines 47 to 50, if that sentence were introduced by the words "Any one of the compounds of Examples 1 to 15 is also readily converted into compounds of the C_A type . . . or "Any one of the herein specifically named cephalosporin C compounds is also readily converted into compounds of the C_A type . . ."

⁴ *In re Ruschig*, 52 CCPA 1238, 343 F.2d 965, 145 USPQ 274 (1965); *In re Kalin*, 54 CCPA 1466, 378 F.2d 959, 154 USPQ 10 (1967); *In re McLa-more*, 54 CCPA 1544, 379 F.2d 985, 154 USPQ 144 (1967); and *In re Ruschig*, 54 CCPA 1551, 379 F.2d 990, 154 USPQ 118 (1967) (*Ruschig II*).

on this point.⁵ There this court reversed the board, finding support for process claims reciting the use of sodium carbonate although the example in the specification advanced as support for the claims used sodium hydroxide. However, in the first place, the *Armstrong* case was decided well before the line of cases beginning with *Ruschig II*, *supra*,⁶ which have significantly tightened up on the application of the description requirement in the first paragraph of 35 U.S.C. 112, and, in the second place, the opinion in *Armstrong* points out that appellants' specification stated that alkali hydroxides and alkali carbonates could be used "interchangeably" in their process. The opinion stresses this equivalency, which involved a tiny number of variables in comparison to the situation here. There are no equivalent "blaze marks," to quote the language of *Ruschig II*, in the case at hand.

Accordingly, we will not sustain the rejection on the ground on which it was made. Concerning the rejection as it is reformulated by the dissent, we express no opinion. It may be that the Patent Office *should* have relied upon the portions of Flynn on which the dissent relies, or it may be that they had very good reasons for not doing so. In any event, they did *not* rely on those teachings in Flynn, and appellants have therefore had no opportunity to comment thereon. We do not conceive that it is part of our duty to make better rejections for the Patent Office, even if we could be sure that we really were making a "better rejection," nor do we think that it would be consistent with the requirements of due process for us to do so for the first time on appeal, without notice to the affected party.

[2] Furthermore, we point out that we are not granting appellants a patent, if that is what the dissent means by "bestowing on the applicants a license to litigate." We are simply reversing a rejection on the ground that the claim on appeal is *anticipated* under § 102 by Flynn. It may well be that it is unpatentable because *obvious* under § 103 in view of Flynn, [3] but no such rejection is before us. The Patent Office is free to make such a rejection after our decision in this case should it think it appropriate. *In re Ruschig*, 54 CCPA 1551, 379 F.2d 990, 154 USPQ 118 (1967); and *In re Fisher*, 58 CCPA 448, 379 F.2d 1406, 171 USPQ 292 (1971). In any event, it is the Patent Office which grants patents, not this [4] court. It may further be observed that

⁵ Among the most recent of these are *In re Ahlbrecht*, 58 CCPA 848, 435 F.2d 908, 911, 168 USPQ 293, 296 (1971); *In re Lukach*, 58 CCPA 1233, 442 F.2d 967, 969, 169 USPQ 795, 796 (1971); and *Fields v. Conover*, 58 CCPA 1366, 443 F.2d 1386, 1391-92, 170 USPQ 276, 279-80 (1971).

it is not now the practice in this court, if it ever was, to apply a different standard in cases which are in "complex areas of technology" than we do in easily understood cases.

The decision of the board is reversed.

BALDWIN, Judge, concurring, with whom ALMOND, Judge, joins.

While I agree that the disclosure in the Flynn patent is insufficient to constitute an anticipation of the claimed invention, I cannot agree with the language of the principal opinion that for the rejection based on an anticipation to have been proper, "the Flynn reference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without any need for picking, choosing, and combining various disclosures not directly related to each other by the teachings of the cited reference."

The test which determines whether an invention has been anticipated by a reference is whether the description of the invention in the reference is "sufficient to put the public in possession of the invention." *In re LeGrice*, 49 CCPA 1124, 1131, 301 F.2d 929, 933, 133 USPQ 365, 369 (1962), citing *Curtis on Patents*, 3d ed., Sec. 378 and *Seymore v. Osborne*, 78 U.S. (11 Wall.) 516, 555 (1870). See also *In re Brown*, 51 CCPA 1254, 329 F.2d 1006, 141 USPQ 245 (1964); *In re Sheppard*, 52 CCPA 859, 339 F.2d 238, 144 USPQ 42 (1964); *In re Bird*, 52 CCPA 1290, 344 F.2d 979, 145 USPQ 418 (1965); *In re Borst*, 52 CCPA 1398, 345 F.2d 851, 145 USPQ 554 (1965); *In re Baranauckas*, 55 CCPA 1204, 395 F.2d 805, 158 USPQ 24 (1968); *In re Hoeksema*, 55 CCPA 1493, 399 F.2d 269, 158 USPQ 596 (1968); *In re Wilder*, 57 CCPA 1314, 429 F.2d 447, 166 USPQ 545 (1970); and *In re Moore*, 58 CCPA 1341, 444 F.2d 572, 170 USPQ 260 (1971). I find it unreasonable to assume that Judge Rich and Judge Lane intend to overrule this long line of cases sub silentio. If what they intend is merely to rephrase the accepted test so as to simplify its application, they have missed the mark.

The language used in the principal opinion would not in fact simplify the determination of the suitability of a reference as an anticipation under 35 U.S.C. 102. That language requires the tribunal to analyze the teachings of a reference to determine which are equivocal and which are unequivocal. It must also be determined which disclosures are directly related to each other by the teachings of the reference, thus making picking and choosing proper, and which disclosures are only indirectly related, or are not related at all. This is no simpler than reading the reference as a whole and determining what it fairly teaches to one of ordinary skill in the art.

The more important difficulty with the position taken in the principal opinion is that it misdirects the inquiry. It directs the tribunal to analyze the structure of the reference rather than its content. The real question is not how logically the various disclosures in a reference are related to each other, it is rather *what the reference fairly teaches to one of ordinary skill in the art*, no matter how ineptly it does so. Of course, the more logically the reference is laid out the clearer will be its teachings and the easier will be the job of those who must interpret it. But the law requires us to determine whether the invention has been *identically* described, *not* whether it has been *logically* described by the reference.

The Flynn reference has been described in both the principal opinion and the dissent. I will therefore merely state what I would consider that reference fairly teaches to one of ordinary skill in the art. Flynn does disclose the cephalosporin C_A-type precursor of the instantly claimed C_A-type compound. The precursor is one of approximately 38 C-type compounds specifically disclosed. Flynn teaches how C-type compounds can be converted to C_C-type compounds by heating with water under acid conditions, or converted to C_A-type compounds by refluxing in an aqueous solution with an excess of a tertiary amine. Pyridine is specifically referred to as an example of a tertiary amine which will work, but a list of over 15 other tertiary amines is given. With regard to antibacterial effect, Flynn discloses that C_C-type compounds are not as good as C-type compounds, and C-type compounds are not as good as C_A-type compounds. As pointed out by the dissent, Flynn considered the C_C-type and C_A-type analogues of the specifically disclosed C-type compounds to be some of the compounds "available in accordance with the present invention."

I would not place as much weight as the dissent does on Flynn's statement that the C_C-type and C_A-type analogues were considered within the scope of the invention. Such statements in the specification regarding the breadth of the invention are generally too speculative to be given great weight. In the instant case, all that statement does is focus some additional attention on C_C-type compounds and C_A-type compounds. In my view, that attention is not a significant addition to the disclosure, since Flynn's remarks regarding the antibacterial activity of the compounds are sufficient to emphasize the C_A-type compounds as the most desirable. The difficulty is that Flynn gives 38 or so possible precursors and 15 or so tertiary amines which will react with those precursors to form C_A-

type compounds. The Flynn disclosure, considered as a whole, does not sufficiently direct one skilled in the art to the claimed compound.

I disagree with the principal opinion on one last point. The opinion seems to suggest that we violate due process whenever we consider portions of a reference not specifically mentioned by the examiner or the board. I know of no requirement that the examiner and the board must list the sentences in the reference upon which they rely, nor can I see any sense in imposing such a requirement. All of the disclosure of a reference must be considered for what it fairly teaches one of ordinary skill in the art. In re Meinhardt, 55 CCPA 1000, 1004, 392 F.2d 273, 276, 157 USPQ 270, 272 (1968). As Judge Smith aptly stated in Meinhardt:

[T]he board relied on the same [reference] as the examiner to sustain the rejection. Assuming arguendo that the board relied on a portion of the [reference] ignored by the examiner, this could not constitute a new ground of rejection in view of In re Azorlosa, 44 CCPA 826, 241 F.2d 939, 113 USPQ 156 (1957), which holds, in pertinent part, that it is proper for the court and necessarily, the board, to consider everything that a reference discloses.

In re Meinhardt, supra, 55 CCPA at 1008-09, 392 F.2d at 280, 157 USPQ at 275. See also In re Halley, 49 CCPA 793, 296 F.2d 774, 132 USPQ 16 (1961); In re Van Mater, 52 CCPA 1076, 341 F.2d 117, 144 USPQ 421 (1965).

WORLEY, Chief Judge, dissenting.

I cannot agree with the majority that cephaloridine is not "described" in the Flynn patent in the sense of 35 U.S.C. 102(e).

It cannot be said, of course, that cephaloridine per se is explicitly named by Flynn, but a clear implicit description is sufficient. In re Baranauckas, 43 CCPA 727, 228 F.2d 413, 108 USPQ 226 (1955). Reference to the Flynn disclosure will establish, I submit, that such a description exists in the present instance.

The principal opinion has set forth portions of the generic and more specific disclosure of Flynn relied on by the board. The class of cephalosporin compounds disclosed generically by Flynn may be divided into several groups, of which the groups designated as cephalosporin C type and cephalosporin C_A type (cephaloridine is a C_A type) are of particular interest here.¹ After observing that "in

¹For purposes here, cephalosporin C_A type compounds differ from cephalosporin C type compounds in the R¹ substituent attached to the methyl group located at the 3 position of the basic cephalosporin (cephem) nucleus. The C_A type

general, those compounds which possess the cephalosporin C_A nucleus are more effective antibacterially than those containing the cephalosporin C nucleus." Flynn goes on to name and describe several specific compounds having the cephalosporin C nucleus:

The following examples, together with the [15] operating examples appearing hereinafter, will illustrate the types of compounds available in accordance with the present invention:

[There follows a list of 24 specific 7-acylamidocephalosporanic acids, i.e., cephalosporin C type compounds. As noted by the board, two of the 15 operating examples referred to, examples 4 and 10, describe the potassium and sodium salts of 7-(2'-thienyl-acetamido) cephalosporanic acid (the sodium salt is known commercially as "cephalothin"). Appellant reacts that particular cephalosporanic acid with the tertiary amine pyridine to obtain the claimed cephalosporin C_A type compound, cephaloridine.]

and the like, including the cephalosporin C_A and cephalosporin C_C analogues thereof. [Emphasis supplied.]

There can be no doubt from the above disclosure that Flynn regarded the cephalosporin C_A analogues of each of the mentioned cephalosporin C type compounds to form an integral part of his disclosed invention. In particular, it is evident that Flynn does explicitly disclose the cephalosporin C_A analogues of Examples 4 and 10. As to how to obtain those C_A analogues from cephalosporin C type compounds, he states that compounds of the cephalosporin C_A class "can be obtained by applying to appropriate 7-acylamidocephalosporanic acids the conversion procedures of Belgian Patent 593,777." Flynn had earlier stated, as pointed out by the board and majority here, just what those "conversion procedures" are, viz., that "Cephalosporin C is also readily converted into compounds of the cephalosporin C_A type by refluxing in aqueous solution with an excess of pyridine, for example, as described in Belgian Patent 593,777."² [Emphasis supplied.]

compounds have a tertiary amine attached to that methyl group, whereas the C type compounds have an acyloxy group so attached. See the formula and definitions under "The Rejection" portion of the principal opinion. Cephaloridine has a pyridine radical attached to the 3-methyl group.

²Belgian 593,777 does indeed disclose obtaining of "antibiotic substances which are transformation products of Cephalosporin C and are called

I think it is clear that Flynn directs one of ordinary skill in the art, who is interested in particular cephalosporin C_A analogues of the 37 or so cephalosporin C type compounds Flynn specifically discloses, to prepare them by reacting the appropriate 7-acylamido cephalosporanic acid with the particular tertiaryamine pyridine. Following those instructions, one of ordinary skill in this art would easily prepare the C_A (pyridine) analogue of the particular cephalosporin C type compound described in Examples 4 and 10, which analogue is cephaloridine. Each and every one of the C_A (pyridine) analogues of that relatively small number of cephalosporin C compounds has been effectively, or implicitly, described by Flynn. To be sure, appellant is claiming only one of them, but it is no less described than any of the others.

From what has been said of Flynn, it should be evident that there is no need in this case for those skilled in the art to resort to picking and choosing various disclosures unrelated to each other by the reference teachings, as the principal opinion implies. On the contrary, the disclosures of cephalosporin C compounds, cephalosporin C_A compounds, and how to make them are all interrelated by Flynn himself. It should also be evident that the reference itself contains the full equivalent of the board's "postulations", which are quoted in footnote 3 and later deprecated in the principal opinion. Finally, it should be evident that the rejection rationale as stated herein is substantially identical to—not a reformulation of—that expressed by the board.

The principal opinion also criticizes the board for reading into references "things that are not there." My difficulty with that position stems from its disregard for the "things"—or "blaze marks"—that are there. In my opinion, the majority is groping for reversible error where none exists. As far back as 40 years, and over the years since, it has been a firm principle that this court would not reverse decisions of the tribunals below in highly complex areas of technology unless manifest error was shown. See, e.g., *In re Wietzel*, 17 CCPA 1079, 39 F.2d 669, 5 USPQ 177 (1930); *In re Bertsch*, 30 CCPA 813, 132 F.2d 1014, 56 USPQ 379 (1942); *In re Stoll*, 34 CCPA 1058, 161 F.2d 241, 73 USPQ 440 (1947). Needless to say, such error has not been shown here.

Although the majority would undoubtedly disclaim the notion, I cannot help but feel that

Cephalosporin C_A compounds" by treatment of Cephalosporin C in aqueous solution with a weak tertiary base, for example pyridine, collidine or quinoline. If pyridine is used, the antibiotic obtained is called Cephalosporin C_A (pyridine)."

it is resolving doubt on the issue presented in favor of the applicants. In doing so, this court is not doing the applicants or the public any favor. Rather it is bestowing on the applicants a license to litigate of dubious validity at a time when, it is reliably estimated, 80% of contested patents are being held invalid in other federal courts. And the other sad result here is to take from the public that which is already theirs by imposing on them a monopoly that should not exist. Appellants have given the public nothing it had not already been given by Flynn. I would remind my colleagues that patents are not like party favors to be passed out at random. The enabling statutes established under the Constitution clearly require more than appellants have offered as a quid pro quo to the public in exchange for the monopoly the majority awards them.

I find no error in the board's decision, and would affirm.

Court of Customs and Patent Appeals

In re MANTELL, SMITH, GALIANO, AND RANKIN

No. 8577

Decided Feb. 17, 1972

PATENTS

Particular patents—Formaldehyde

Mantell, Smith, Galiano, and Rankin, Formaldehyde Block Copolymers and Processes, claims 6, 16, and 18 of application allowed; claims 1 and 3 refused.

Appeal from Board of Appeals of the Patent Office.

Application for patent of Gerald J. Mantell, Wayne E. Smith, Francis R. Galiano, and David Rankin, Serial No. 313,192, filed Oct. 2, 1963; Patent Office Group 140. From decision rejecting claims 1, 3, 6, 8, 9, 11, 12, 16, and 18, applicants' appeal. Affirmed as to claims 1 and 3; reversed as to claims 6, 16, and 18; remanded as to claims 8, 9, 11, and 12.

WILLIAM H. DRUMMOND, Phoenix, Ariz., ERIC P. SCHELLIN, Arlington, Va., and RICHARD L. KELLY, Kansas City, Mo., for appellants.

S. WM. COCHRAN (FRED W. SHERLING, of counsel) for Commissioner of Patents.

PCT

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(51) 国際特許分類6 A61K 38/18, 9/14, 47/02, 47/10, 47/12, 47/18, 47/36	A1	(11) 国際公開番号 WO97/02832 (43) 国際公開日 1997年1月30日(30.01.97)
(21) 国際出願番号 PCT/JP96/01898 (22) 国際出願日 1996年7月8日(08.07.96) (30) 優先権データ 特願平7/199018 1995年7月11日(11.07.95) JP (71) 出願人 (米国を除くすべての指定国について) 雪印乳業株式会社 (SNOW BRAND MILK PRODUCTS CO., LTD.)(JP/JP) 〒065 北海道札幌市東区苗穂町6-1-1 Hokkaido, (JP) 住友製菓株式会社 (SUMITOMO PHARMACEUTICALS CO., LTD.)(JP/JP) 〒541 大阪府大阪市中央区道修町2-2-8 Osaka, (JP) (72) 発明者: および (75) 発明者/出願人 (米国についてのみ) 田中克実(TANAKA, Katsumi)(JP/JP) 〒569 大阪府高槻市玉川1-9-1 住友化学高槻社宅110 Osaka, (JP) 東尾侃二(HIGASHIO, Kanji)(JP/JP) 〒350 埼玉県川越市山田1769-10 Saitama, (JP)	(74) 代理人 弁理士 廣瀬孝美(HIROSE, Takayoshi) 〒530 大阪府大阪市北区西天満5丁目13番3号 高橋ビル北3号館6階 Osaka, (JP) (81) 指定国 AU, CA, KR, US, 欧州特許 (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). 添付公開書類 国際調査報告書	
(54) Title: LYOPHILIZED HGF PREPARATIONS (54) 発明の名称 HGF凍結乾燥製剤 (57) Abstract A lyophilized HGF preparation prepared by lyophilizing an aqueous HGF solution, and a lyophilized HGF preparation further containing a stabilizer, sodium chloride, a buffer and/or a surfactant, or other additive(s). The lyophilized preparations can stabilize HGF and enables long-term storage.		

(57) 要約

本発明は、HGFを含有する水溶液を凍結乾燥したHGF凍結乾燥製剤、及び安定化剤、塩化ナトリウム、緩衝剤及び／又は界面活性剤等を添加したHGF凍結乾燥製剤に関する。本発明によれば、HGFを安定化させることができ、長期間の保存が可能となった。

情報としての用途のみ

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CU	キューバ	KR	大韓民国	NO	ノルウェー	UZ	ウズベキスタン
CZ	チェッコ共和国	KZ	カザフスタン	NZ	ニュー・ジーランド	VN	ヴィエトナム

明 細 書

HGF凍結乾燥製剤

5 技術分野

本発明は、HGF (Hepatocyte Growth Factor)を含有する溶液を凍結乾燥することにより得られる、HGF凍結乾燥製剤に関する。さらに詳しくは、安定化剤、塩化ナトリウム、緩衝剤、又は界面活性剤の少なくとも一種以上を含有する、前記HGF凍結乾燥製剤に関する。本発明により、HGFを安定化させた、長期保存の可能な製剤が提供される。

背景技術

HGFは肝実質細胞の増殖活性を有する蛋白質であり、異なったアミノ酸配列を有するものが報告されており、その名称も、HGF、TCF、SCF等が使用されている。本発明では、これらの公知の肝実質細胞増殖活性を有する蛋白質をHGFと総称する。

HGFは様々な薬理作用を示す生理活性ペプチドであり、その薬理作用については、例えば実験医学 Vol.10, No.3 (増刊) 330-339 (1992)に記載されている。HGFはその薬理作用から肝硬変治療剤、腎疾患治療剤、上皮細胞増殖促進剤、抗ガン剤、ガン療法用副作用防止剤、肺障害治療剤、胃・十二指腸損傷治療剤、脳神経障害治療剤、免疫抑制副作用防止剤、コラーゲン分解促進剤、軟骨障害治療剤、動脈疾患治療剤、肺線維症治療剤、肝臓疾患治療剤、血液凝固異常治療剤、血漿低蛋白治療剤、創傷治療剤、神経障害改善薬、造血幹細胞増加剤、育毛促進剤等(日本特開平4-18028号公報、日本特開平4-49246号公報、EP 492614号公報、日本特開平6-25010号公報、W0 93/8821号公報、日本特開平6-172207号公報、日本特開平7-89869号公報、日本特開平6-40934号公報、W0 94/2165号公報、日本特開平6-40935号公報、日本特開平6-56692号公報、日本特開平7-41429号公報、W0 93/3061号公報、日本特開平5-213721号公報等)としての開発が期待されている。

HGFの製剤については、W0 90/10651公報及び日本特開平6-247872号公報に記載がある。上記W0 90/10651公報は、HGFと比較してアミノ酸5残基が欠失したデリ

ーションタイプのHGF (dLeHGF) について開示されており、TCFIIと称している。この明細書では、アルブミン、ヒト血清、ゼラチン、ソルビトール、マンニトール、キシトール等がHGFを安定化することを開示している。これらは、水溶液製剤に関するものであり、HGFを水溶液中で安定化させる。また、日本特
5 開平6-247872号公報は塩基性アミノ酸等とHGF (TCF) を共存させることにより、HGFを高濃度に含有させた製剤について開示している。

ところで、タンパク質は一般に凍結操作においてそれほど安定ではない（「蛋白質 核酸 酵素」 37(9), 1517 (1992)）。また、水溶液中におけるタンパク質の安定化剤は、水分子とタンパク質との相互作用によって安定化させるものであり、従っ
10 て、水の存在しないタンパク質の凍結乾燥品においては、水溶液におけるタンパク質の安定化剤は、多くの場合、安定化効果を示さない（「蛋白質 核酸 酵素」 37(9), 1517 (1992)）。

一方、HGFの凍結乾燥製剤についてはまったく知られておらず、またHGFの凍結乾燥製剤がどの程度の物理的及び生物活性的安定性を示すかは予想することが
15 でなかった。

HGFの水溶液製剤自体は、低温又は室温で数日間保存すると、凝集、白濁、ゲル化が認められ、性状が変化し、類縁体・重合体が形成される等、物理的安定性が低く、また生物活性が低下する等、生物活性安定性が低く、長期間の保存に対し安定な製剤ではない。そのことは、HGFを注射用製剤等とした医薬又は動物薬とし
20 ての開発に大きな障害となっていた。本発明は上記の従来課題を解決するものである。即ち、本発明の目的は、医療用医薬品又は動物用薬として長期間の保存でも安定な製剤の提供にある。

発明の開示

25 本発明は、HGF凍結乾燥製剤である。当該HGF凍結乾燥製剤は、グリシン、アラニン、ソルビトール、マンニトール、デキストラン硫酸などの安定化剤を含有していてもよく、またクエン酸塩などの緩衝剤を含有していてもよい。

また、本発明の他の発明は、安定化剤、塩化ナトリウム、緩衝剤及び界面活性剤を含有するHGF凍結乾燥製剤である。

30 本発明のHGF凍結乾燥製剤においては、HGFが安定化され、長期保存が可能

となる。

発明を実施するための最良の形態

5 本発明に使用されるHGFとしては、医薬として使用できる程度に精製されたものであれば、種々の方法で精製されたものを用いることができる。

HGFの精製方法としては、各種の方法が知られている。例えば、ラット、ウシ、ウマ、ヒツジなどの哺乳動物の肝臓、脾臓、肺臓、骨髓、脳、腎臓、胎盤等の臓器、血小板、白血球等の血液細胞や血漿、血清などから抽出、精製して得ることができる(FEBS Letters, 224, 312, 1987、Proc. Natl. Acad. Sci. USA, 86, 5844, 1989など参照)。

また、HGFを産生する初代培養細胞や株化細胞を培養し、培養物(培養上清、培養細胞等)から分離精製してHGFを得ることもできる。あるいは遺伝子工学的手法によりHGFをコードする遺伝子を適切なベクターに組み込み、これを適当な宿主に挿入して形質転換し、この形質転換体の培養上清から目的とする組換えHGF
15 を得ることができる(例えば、Nature, 342, 440, 1989、WO 92/01053公報、日本特開平5-111383号公報、Biochem. Biophys. Res. Commun., 163, 967, 1989など参照)。上記の宿主細胞は特に限定されず、従来から遺伝子工学的手法で用いられている各種の宿主細胞、例えば大腸菌、枯草菌、酵母、糸状菌、植物又は動物細胞などを用いることができる。

20 より具体的には、HGFを生体組織から抽出精製する方法としては、例えば、ラットに四塩化炭素を腹腔内投与し、肝炎状態にしたラットの肝臓を摘出して粉碎し、S-セファロース、ヘパリンセファロースなどのゲルカラムクロマトグラフィー、HPLC等の通常の蛋白質精製法にて精製することができる。

また、遺伝子組換え法を用い、ヒトHGFのアミノ酸配列をコードする遺伝子を、
25 ウシパピローマウィルスDNAなどのベクターに組み込んだ発現ベクターによって動物細胞、例えば、チャイニーズハムスター卵巣(CHO)細胞、マウスC127細胞、サルCOS細胞などを形質転換し、その培養上清より得ることができる。

かくして得られたHGFは、肝細胞の増殖効果を有していれば、そのアミノ酸配列の一部が欠失又は他のアミノ酸により置換されていたり、他のアミノ酸配列が一部挿入されていたり、N末端及び／又はC末端に1又は2以上のアミノ酸が結合し
30

ていたり、あるいは糖鎖が同様に欠失又は置換されていてもよい。

「HGF凍結乾燥製剤」とは、HGFを含有する水溶液を通常の凍結乾燥方法で凍結乾燥した製剤をいう。

「安定化剤」としては、グリシン、アラニン等のアミノ酸類、ヘパリン、デキス
5 トラン硫酸等の多糖類、ソルビトール、マンニトール等の糖アルコール等が挙げられ、二種以上を併用してもよい。安定化剤を加えて製造したHGF凍結乾燥製剤は、さらにHGFの保存安定性が増した製剤である。好ましい安定化剤は、グリシン、アラニン、ソルビトール、マンニトール、デキストラン硫酸等が挙げられる。例えば、グリシン、アラニン、ソルビトール又はマンニトールの添加量として好ましい
10 のは、HGFの重量に対して、0.01-100倍の重量が挙げられ、特に好ましいのは、0.1-10倍の重量が挙げられる。

「緩衝剤」としては、例えばリン酸バッファー、クエン酸バッファー等が挙げられる。緩衝剤は、再溶解後の水溶液のpHを調整しHGFの溶解性を保つ作用を有する。すなわち、例えば実施例で使用した組換HGFの場合、pHによってHGF
15 の溶解度は変化し、pH7付近では0.1-5.0mg/mlの溶解度を示すが、pH5付近では20mg/ml以上の溶解度を示すため、pHを5.0-6.0にするのが好ましい。緩衝剤として好ましいものは、クエン酸バッファーが挙げられ、特に好ましくはクエン酸ナトリウムバッファーが挙げられる。このクエン酸バッファーは、再溶解後の水溶液中でのHGFの安定化にも寄与する。緩衝剤の添加量として好ましい範囲は、例えば再溶解後の水量に対し、1-100mMとなる範囲が
20 挙げられる。

「界面活性剤」としては、例えばポリソルベート20、ポリソルベート80、ブルロニックF-68、ポリエチレングリコール等が挙げられ、二種以上を併用してもよい。界面活性剤として特に好ましくは、ポリソルベート80を挙げることができる。HGFは容器の材質であるガラスや樹脂などに吸着しやすい。従って、界面
25 活性剤を添加することによって、再溶解後のHGFの容器への吸着を防止することができる。界面活性剤の添加量として好ましい範囲は、例えば再溶解後の水重量に対し、0.001-2.0%の重量の範囲が挙げられる。

「塩化ナトリウム」はHGFの溶解性を保つ作用を有する。すなわち、例えば実施
30 例で使用した組換HGFの場合、塩化ナトリウムの添加により溶解度が向上し、

特に 300 mM 以上では著しく溶解性が向上する（日本特開平6-247872号公報）。塩化ナトリウムの添加量は浸透圧比により制限を受けるが、一般的に用いられる注射剤の浸透圧比を示す量でよい。特に医療用又は動物薬用注射剤の浸透圧比として許容される浸透圧比 1 - 2 が好ましく、例えば再溶解後の水量に対し 150 - 300 mM とすることが好ましい。

HGF 凍結乾燥製剤は、HGF を含有する水溶液を通常の凍結乾燥方法で凍結乾燥することで製造できる。例えば、HGF を適切な溶剤（例えば、滅菌水、緩衝液、生理食塩水等）に溶解した後、フィルター等で濾過して滅菌し、必要に応じて、安定化剤、緩衝剤、界面活性剤、塩化ナトリウム等を加え、凍結乾燥する。本発明の製剤は製剤化に必要な添加物、例えば、溶解補助剤、酸化防止剤、無痛化剤、等張化剤等を含んでもよい。凍結乾燥方法としては、例えば、①常圧下で冷却凍結する凍結過程、②溶質に拘束されない自由水を減圧下で昇華乾燥する 1 次乾燥過程、③溶質固有の吸着水や結晶水を除去する 2 次乾燥過程の 3 つの単位操作による方法が挙げられる（Pharm. Tech. Japan, 8(1), 75-87 (1992)）。HGF は、溶液調製時、凍結乾燥時、及びその凍結乾燥製剤を再溶解した水溶液において、非常に安定である。なお、HGF 含量は、適用疾患、適用投与経路などに応じて適宜調整することができる。

凍結乾燥製剤は、使用時に注射用蒸留水等を加え、再溶解して使用される。

20 産業上の利用可能性

本発明の HGF 凍結乾燥製剤は、HGF を安定化させることができ、長期間の保存が可能となった。

実施例

25 以下、実施例を挙げて本発明をさらに詳細に説明するが、本発明はこれらの実施例によりなんら限定されるものではない。なお、本実施例においては、WO 90/10651 公報に記載の dLeHGF（5 アミノ酸欠失型 HGF、別名 TCFII）を用いた。

実施例 1

30 HGF 凍結乾燥製剤の作製

300 mM塩化ナトリウム、0.01%ポリソルベート80を含有する10 mMクエン酸緩衝液 (pH 5.0) にHGF 20 mg/mlとなるように溶解し、無菌的にHGF水溶液を得た。本水溶液のpHを調整した後、無菌的にバイアルに充填し、表1に示す条件に従って凍結乾燥して、HGF凍結乾燥製剤を得た。なお、表

表1

	凍結過程		1次乾燥過程		2次乾燥過程	
温度 (°C)	5 → -40	-40	-40 → 0	0	0 → 20	20
時間 (hr)	1	10	8	24	1	24
圧力 (mmHg)	760	760	<1	<1	<1	<1

実施例2

HGF凍結乾燥製剤の作製

実施例1において、10 mMクエン酸緩衝液 (pH 5.0) の代わりに10 mMクエン酸緩衝液 (pH 6.0) を用いて、HGF凍結乾燥製剤を得た。

実施例3

HGF凍結乾燥製剤の作製

実施例1において、10 mMクエン酸緩衝液 (pH 5.0) の代わりに10 mMリン酸緩衝液 (pH 6.0) を用いて、HGF凍結乾燥製剤を得た。

実施例4

HGF凍結乾燥製剤の作製

実施例1において、10 mMクエン酸緩衝液 (pH 5.0) の代わりに10 mMリン酸緩衝液 (pH 7.0) を用いて、HGF凍結乾燥製剤を得た。

実施例5

HGF凍結乾燥製剤の作製

300 mM塩化ナトリウム、0.01%ポリソルベート80を含有する10 mMクエン酸緩衝液 (pH 5) にHGF 20 mg/mlとなるように溶解した。続いて、

グリシンを50 mg/mlになるよう溶解し、無菌的にHGF溶解液を得た。本溶解液のpHを調整した後、無菌的にバイアルに充填し、実施例1の凍結乾燥の条件と同様の条件によりHGF凍結乾燥製剤を得た。

5 実施例6

HGF凍結乾燥製剤の作製

実施例5において、グリシンの代わりにアラニンを用いて、HGF凍結乾燥製剤を得た。

10 実施例7

HGF凍結乾燥製剤の作製

300 mM塩化ナトリウム、0.01%ポリソルベート80を含有する10 mMクエン酸緩衝液(pH5)にHGF20 mg/mlとなるように溶解した。続いて、ソルビトールを200 mg/mlになるよう溶解し、無菌的にHGF溶解液を得た。
15 本溶解液のpHを調整した後、無菌的にバイアルに充填し、実施例1の凍結乾燥の条件と同様の条件によりHGF凍結乾燥製剤を得た。

実施例8

HGF凍結乾燥製剤の作製

20 300 mM塩化ナトリウム、0.01%ポリソルベート80を含有する10 mMクエン酸緩衝液(pH6)にHGF10 mg/mlとなるように溶解した。続いて、デキストラン硫酸を50 mg/mlになるよう溶解し、pHを調整して、HGF溶解液を得た。次いで、バイアル充填し、実施例1の凍結乾燥の条件と同様な条件によりHGF凍結乾燥製剤を得た。

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実施例9

HGF凍結乾燥製剤の作製

実施例1において、10 mMクエン酸緩衝液(pH5.0)の代わりに10 mMクエン酸緩衝液(pH6.0)を用い、またHGF濃度を10 mg/mlとして、
30 HGF凍結乾燥製剤を得た。

試験例 1

HGF の生物活性に及ぼす凍結乾燥過程の影響

凍結乾燥製過程における HGF の生物活性の変化を確認するため、実施例 1 に
 いて、凍結乾燥前の HGF 水溶液及び凍結乾燥後そのまま再溶解した HGF 水溶液
 を用いて HGF の生物活性を測定した（生物活性測定法は以下に示す）。その結果
 を表 2 に示す。凍結乾燥前後で比活性に変化が認められなかったことから、凍結乾
 燥過程及び再溶解において HGF の生物活性は失活せず、HGF を凍結乾燥製剤と
 することが可能であることが示された。

生物活性測定方法

Wistar 系雄性ラットを肝灌流して得られた肝細胞を精製し、細胞生存率を
 確認後、 1×10^4 / well でプレートに播種した。5%炭酸ガスインキュベータ
 でのブレインキュベーション 20 時間後、HGF サンプル及び標準品を添加した（
 $n=3$ ）。さらに、5%炭酸ガスインキュベータでのブレインキュベーション 24
 時間後、 $[^3\text{H}]$ -チミジンを添加し、2 時間ラベルした。細胞をセルハーベスター
 で回収し、細胞内に取り込まれた $[^3\text{H}]$ 量を測定した。測定結果を平行線検定法に
 かけ、標準品に対する比活性を求めた。

表 2

凍結乾燥前後における生物活性	
サンプル	比活性
凍結乾燥前 溶液製剤	0.89
凍結乾燥製剤 溶解直後	0.94

試験例 2

凍結乾燥製剤溶解後の性状

実施例で作製した凍結乾燥製剤を、 -40°C 、 25°C 、 50°C にて 1 ヶ月間保存
 後、溶解し、溶解後の性状を目視により観察した。凍結乾燥製剤の溶解は精製水で
 行った。その結果を表 3 に示す。 -40°C 及び 25°C の保存において、いずれの実
 施例の製剤も性状に関して安定であった。また、 50°C の保存においては、実施例
 1 の製剤は溶解直後白濁したが、実施例 5、6 及び 7 の製剤は性状に関して安定で

あった。

表 3

凍結乾燥製剤溶解後の性質(1ヶ月保存品)			
製剤	性 状		
	-40℃	25℃	50℃
実施例1	澄明	澄明	白濁
実施例5	澄明	澄明	澄明
実施例6	澄明	澄明	澄明
実施例7	澄明	澄明	澄明

試験例 3

凍結乾燥製剤における重合体含量変化

実施例1、5、6及び7で作製した凍結乾燥製剤を、-40℃、25℃、40℃、50℃にて1ヶ月間又は2ヶ月間保存し、その凍結乾燥製剤に含まれる重合体含量とHGF含量の比を測定した。測定方法は以下に示すゲルろ過法を使用した。その結果を表4及び表5に示す。いずれの温度の保存においても、いずれの実施例の製剤も重合体の生成は少なく物理的に安定であった。また、特に実施例5、6及び7の製剤は重合体の生成は極端に少なく物理的に安定であった。

重合体含量測定方法

HGF濃度を2mg/mlに希釈後、ゲル濾過法を用いて、下記の条件で測定した。

カラム : TOSOH TSK G-3000SW XL ($\phi 0.78 \times 30\text{cm}$)

流速 : 0.5 ml/min

検出 : OD 280

温度 : 25 °C

キャリアー : 10mM Tris, 150mM NaCl, 0.05% SDS, pH 7.0

アプライ : 20 μl

重合体の保持時間 : 13.0 min

HGFの保持時間 : 14.4 min

表4

1ヶ月保存の凍結乾燥製剤の重合体含量/HGF含量				
	-40℃	25℃	40℃	50℃
実施例1	1.07 %	1.59 %	2.76 %	6.17 %
実施例5	0.92 %	1.39 %	1.83 %	4.09 %
実施例6	0.93 %	1.54 %	1.81 %	2.90 %
実施例7	0.90 %	1.35 %	2.57 %	6.64 %

表5

2ヶ月保存の凍結乾燥製剤の重合体含量/HGF含量				
	-40℃	25℃	40℃	50℃
実施例1	0.92 %	1.44 %	3.91 %	12.23 %
実施例5	0.88 %	1.21 %	2.49 %	7.49 %
実施例6	0.85 %	1.10 %	1.96 %	5.76 %

試験例4

重合体生成に及ぼすデキストラン硫酸の影響

実施例8で調製した凍結乾燥製剤を、50℃にて1ヶ月保存し、その凍結乾燥製剤に含まれる重合体含量とHGF含量の比を測定した。なお、測定は試験例3と同様にして行った。また、比較例として、デキストラン硫酸を含まない点以外は同様な成分及び方法により調製されている実施例9の凍結乾燥製剤を用いて、同様な試験を行った。その結果を表6に示す。表6に示されるように、デキストラン硫酸を添加することにより、高温保存しても重合体の生成は少なく、安定性が向上することが判明した。

表6

凍結乾燥製剤の重合体含量/HGF含量		
	保存開始前	50℃, 1ヶ月保存後
実施例8	2.46 %	9.45 %
実施例9	1.78 %	14.01 %

試験例 5

凍結乾燥製剤の生物活性変化

実施例 1、5、6 及び 7 で作製した凍結乾燥製剤を、 -40°C 、 40°C 、 50°C 、 60°C にて1ヶ月間又は2ヶ月間保存し、その凍結乾燥製剤を再溶解した水溶液の生物活性を、試験例 1 に示す生物活性測定方法で測定した。その結果を表 7 及び表 8 に示す。なお、実施例 1、5、6 及び 7 の製剤の再溶解後の水溶液の生物活性の初期値は、それぞれ 1.01 ± 0.25 、 0.91 ± 0.18 、 0.88 ± 0.05 、 1.03 ± 0.04 であった。 60°C の保存では、やや生物活性に低下傾向が見られるものの、 50°C 以下の保存では、いずれの実施例の製剤も生物活性に殆ど変化はなく、生物活性的に安定であった。

表 7

1ヶ月保存の凍結乾燥製剤の生物活性（比活性）				
	-40°C	40°C	50°C	60°C
実施例 1	0.96 ± 0.13	0.92 ± 0.13	0.81 ± 0.07	0.54 ± 0.05
実施例 5	0.80 ± 0.14	0.99 ± 0.10	0.80 ± 0.16	0.72 ± 0.03
実施例 6	0.92 ± 0.14	1.02 ± 0.06	0.94 ± 0.08	0.78 ± 0.03
実施例 7	0.92 ± 0.02	0.97 ± 0.04	0.83 ± 0.06	---

表 8

2ヶ月保存の凍結乾燥製剤の生物活性（比活性）			
	-40°C	40°C	60°C
実施例 1	1.14 ± 0.14	0.98 ± 0.01	0.46 ± 0.09
実施例 5	0.95 ± 0.05	0.84 ± 0.09	0.57 ± 0.01
実施例 6	1.11 ± 0.14	1.09 ± 0.03	0.52 ± 0.02

請求の範囲

1. HGF凍結乾燥製剤。
2. 安定化剤を含有する請求の範囲1記載のHGF凍結乾燥製剤。
- 5 3. 安定化剤がグリシン、アラニン、ソルビトール、マンニトール又はデキストラン硫酸である請求の範囲2記載のHGF凍結乾燥製剤。
4. 緩衝剤を含有する請求の範囲1から3の何れかに記載のHGF凍結乾燥製剤。
5. 緩衝剤がクエン酸塩である請求の範囲4記載のHGF凍結乾燥製剤。
- 10 6. 安定化剤、塩化ナトリウム、緩衝剤及び界面活性剤を含有するHGF凍結乾燥製剤。

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP96/01898

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl ⁶ A61K38/18, 9/14, 47/02, 47/10, 47/12, 47/18, 47/36 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int. Cl ⁶ A61K38/18, 9/14, 47/02, 47/10, 47/12, 47/18, 47/36 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP, 6-40935, A (Snow Brand Milk Products Co., Ltd.), February 15, 1994 (15. 02. 94) & EP, 588477, A	1 - 6
X	JP, 6-40938, A (Toshikazu Nakamura and another), February 15, 1994 (15. 02. 94) (Family: none)	1 - 6
X	JP, 6-172207, A (Toshikazu Nakamura and another), June 21, 1994 (21. 06. 94) (Family: none)	1 - 6
X	JP, 6-247872, A (Snow Brand Milk Products Co., Ltd.), September 6, 1994 (06. 09. 94) & EP, 612530, A & US, 5510327, A	1 - 6
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search September 26, 1996 (26. 09. 96)		Date of mailing of the international search report October 8, 1996 (08. 10. 96)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.		Authorized officer Telephone No.

A. 発明の属する分野の分類 (国際特許分類 (IPC))		
Int. Cl. ⁴ A 61 K 38/18, 9/14, 47/02, 47/10, 47/12, 47/18, 47/36		
B. 調査を行った分野		
調査を行った最小限資料 (国際特許分類 (IPC))		
Int. Cl. ⁴ A 61 K 38/18, 9/14, 47/02, 47/10, 47/12, 47/18, 47/36		
最小限資料以外の資料で調査を行った分野に含まれるもの		
国際調査で使用した電子データベース (データベースの名称、調査に使用した用語)		
C. 関連すると認められる文献		
引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する 請求の範囲の番号
×	J P, 6-40935, A, (雪印乳業株式会社) 15. 2月. 1994 (15. 02. 94) & E P, 588477, A	1-6
×	J P, 6-40938, A, (中村敏一 他) 15. 2月. 1994 (15. 02. 94) (ファミリーなし)	1-6
×	J P, 6-172207, A, (中村敏一 他) 21. 6月. 1994 (21. 06. 94) (ファミリーなし)	1-6
<input checked="" type="checkbox"/> C欄の続きにも文献が列挙されている。 <input type="checkbox"/> パテントファミリーに関する別紙を参照。		
* 引用文献のカテゴリー 「A」特に関連のある文献ではなく、一般的技术水準を示すもの 「E」先行文献ではあるが、国際出願日以後に公表されたもの 「L」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献 (理由を付す) 「O」口頭による開示、使用、展示等に言及する文献 「P」国際出願日前で、かつ優先権の主張の基礎となる出願日の後に公表された文献 「T」国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの 「X」特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの 「Y」特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの 「&」同一パテントファミリー文献		
国際調査を完了した日 26. 09. 96		国際調査報告の発送日 08.10.96
国際調査機関の名称及びあて先 日本国特許庁 (ISA/J P) 郵便番号100 東京都千代田区霞が関三丁目4番3号		特許庁審査官 (権限のある職員) 松 浦 新 司 印
		4 C 8 3 1 4
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C (続き) 関連すると認められる文献		
引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する 請求の範囲の番号
X	J P, 6-247872, A, (雪印乳業株式会社) 6. 9月. 1994 (06. 09. 94) & EP, 612530, A, & US, 5510327, A	1-6

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(71) Applicant: **Nakamura, Toshikazu**
11-6, Midorigaoka 3-chome, Higashi-ku
Fukuoka-shi, Fukuoka 812(JP)

Applicant: **Toyo Boseki Kabushiki Kaisha**
No.2-8, Dojimahama 2-chome Kita-ku
Osaka-shi Osaka 530(JP)

(72) Inventor: **Nakamura, Toshikazu**
11-6, Midorigaoka 3-chome
Higashi-ku, Fukuoka-shi, Fukuoka 812(JP)

(74) Representative: **von Kreisler, Alek,**
Dipl.-Chem. et al
Patentanwälte Von Kreisler-Selting-Werner,
Deichmannhaus am Hauptbahnhof
W-5000 Köln 1(DE)

(54) **Therapeutic agent for hepatocirrhosis.**

(57) A therapeutic agent for hepatocirrhosis containing a hepatocyte growth factor as an active ingredient. Since the hepatocyte growth factor suppresses fiber formation and simultaneously stimulates hepatocyte proliferation, it is considered to be effective for preventing the transition from chronic hepatitis to hepatocirrhosis and progress of hepatocirrhosis, and also for the treatment of considerably advanced hepatocirrhosis and other hepatic diseases such as hepatitis.

EP 0 456 188 A1

The present invention relates to a therapeutic agent for hepatocirrhosis containing a hepatocyte growth factor as an active ingredient.

Liver is an organ indispensable for humans, which has a variety of physiological functions such as gluconeogenesis, amino acid metabolism, lipid metabolism, synthesis and secretion of plasma protein, formation and secretion of bile, detoxication, storage of sugar as an energy source, storage of vitamins and so on. The liver develops hepatitis due to virus infection, long-term intake of alcohol or medicaments, or the like, and hepatitis chronically proceeds to hepatocirrhosis. Patients with hepatocirrhosis die with high probability.

At present, the number of patients with hepatocirrhosis in Japan is estimated to be about 400,000, and about 20,000 patients die thereof annually. It is presumed that 80% of the patients suffer from hepatocirrhosis induced by hepatitis caused by B and non-A, non-B virus infections and the most part of the rest 20% suffer from hepatocirrhosis induced by alcoholic hepatitis.

However, there is practically no therapeutic agents for the treatment of hepatocirrhosis. What is being given to the patients is symptomatic therapy by drip infusion and per rectum infusion of vitamins and amino acid fluids to alleviate hypoalimentation and hypoproteinosis observed along with the progress of hepatocirrhosis. Administrations of glycyrrhizin, glutathione, thiopronin, ATP preparations or extracts from animal livers as liver-protecting agents are also employed, albeit with inconclusive effects.

Meanwhile, *in vitro* cultivation of mature parenchymal cells controlling the liver functions had been unattainable for a long time until the present inventor succeeded in partial purification of a protein component on the basis of the finding that mature hepatocytes proliferate extremely well when the particular protein component contained in regenerating hepatic rat sera is added to the medium [Biochem. Biophys. Res. Commun., 122 (No. 3), 1450-1459, 1984], which was named hepatocyte growth factor (hereinafter sometimes referred to as HGF). In addition, the present inventor succeeded in isolating the hepatocyte growth factor from rat platelets [FFBS LETTER, 22 (No. 2), 311, 1987] and determined part of the amino acid sequence (Nature, 342, 440-443, 1989).

The present inventor further conducted cDNA cloning of human- or rat-originated HGF on the basis of the obtained amino acid sequence of HGF and obtained the hepatocyte growth factor through transfection of said cDNA into animal cells (Nature, 342, 440-443, 1989).

Hepatocirrhosis is understood as an ultimate stage of chronic inflammation and fibrosis of a liver. That is, fiber dissepiments are developed through-

out the liver, forming innumerable pseudolobules. In other words, it is a state of suppression of hepatocyte growth and abnormal hyperplasia of interstitial bindwebs. Where the fiber dissepiments evolve into pseudolobules, hepatocytes are isolated, preventing maintenance of smooth bloodstream and transportation of substances. Moreover, original vessels become shunts passing through the fiber dissepiments, which gives rise to decrease of effective bloodstream. Such abnormality of bloodstream along with the progress of hepatocirrhosis can be a factor for promoting degeneration of the liver, which encourages vicious circle in hepatocirrhosis.

The main cause of hepatocirrhosis is chronic inflammation over a long period, and hyperplasia of bindwebs is considered to be secondary. For this reason, it is primarily necessary to prevent onset of chronic inflammation. In addition, inhibition of fiber formation by way of suppressing fibroblast proliferation and stimulation of hepatocyte growth are considered to be effective for preventing the transition from chronic inflammation to hepatocirrhosis and progress of hepatocirrhosis, which may eventually enable treatment of even hepatocirrhosis in a considerably advanced stage.

It has been expected that a substance having two physiological activities of specifically stimulating proliferation of hepatocytes and specifically suppressing proliferation of hepatic nonparenchymal fibroblasts can be used as an agent for the treatment of hepatocirrhosis, and provision of such agent is aimed at in the present invention.

SUMMARY OF THE INVENTION

The present inventor has conducted intensive studies for the purpose of solving the above-mentioned problems, and as a result, found that HGF which has been identified as a hepatocyte growth-stimulating factor also possesses the activity to suppress rat hepatic nonparenchymal fibroblasts in primary culture, which resulted in completion of the invention.

BRIEF EXPLANATION OF THE DRAWING

Fig. 1 is a graph showing effects of HGF on hepatocytes in primary culture and on hepatic nonparenchymal fibroblasts in primary culture.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to therapeutic agents for hepatocirrhosis, containing HGF as an active ingredient.

The HGF used in the present invention is a physiologically active polypeptide co-possessing

the activity enabling proliferation of hepatocytes and the activity suppressing proliferation of hepatic nonparenchymal fibroblasts, which has a molecular weight of 70,000 to 90,000 by nonreduction polyacrylamide gel electrophoresis. Under reducing conditions, it takes the heterodimer structure comprising α -chain of 60,000 to 75,000 molecular weight and β -chain of 30,000 to 40,000 molecular weight. Based on the amino acid sequence of human HGF and rat HGF, it is speculated that the α -chain has a special sequence called kringle structure as can be seen in plasminogen and plasmin, and the sequence in the β -chain resembles the serine * protease domain such as kallikrein and coagulation factor XII. Since HGF shows affinity to concanavalin A, it is a glycoprotein having a sugar chain therein.

HGF can be obtained by various methods. For example, it can be obtained from organs such as liver, spleen, lung, bone marrow, brain, kidney and placenta, blood cells such as platelet and leukocyte and plasma (including serum) of mammals such as cow by extraction and purification. It is also possible to obtain HGF by cultivation of primary culture cells and strain cells capable of HGF production, followed by separation and purification from the culture. Needless to say, HGF can be obtained by genetic engineering comprising isolation of the HGF gene, transformation of suitable host cells and cultivation of the transformants obtained, followed by isolation-purification of the objective recombinant hepatocyte growth factor from the culture (See Nature, 342, 440-443, 1989).

Recombinant human HGF is prepared by, for example,;

- (1) mRNA or genomic DNA is isolated from animal tissues such as rat hepatocytes or rat megakaryocytes, from which cDNA libraries or genomic DNA libraries are prepared;
- (2) desired cDNAs or genomic DNAs are prepared from isolated clones by screening the above-mentioned animal- (e.g. rat) originated cDNA libraries or genomic DNA libraries for isolation of the cDNA or genomic DNA of HGF of an animal such as rat with synthetic oligonucleotide probes or antibodies, and objective human-originated HGF cDNAs are extracted from the isolated clones by screening the cDNA libraries prepared from mRNA of human organs or blood cells with the rat-originated HGF cDNAs or genomic DNAs as the probe. Also, human-originated HGF cDNAs can be prepared from the isolated clones by screening the cDNA libraries constructed from mRNA extracted from human organs or blood cells. The screening of the cDNA library mentioned can be carried out by using as the probe, the human HGF cDNAs or the human HGF genomic DNAs synthesized

on the basis of the DNA sequence determined by the present inventor or the human or animal HGF amino acid sequence, or by using antibodies against human or animal HGF;

(3) cDNA fragments encoding the human HGF are cleaved from the human-originated HGF cDNAs with restriction enzymes and integrated in the expression vectors;

(4) By the obtained recombinant expression vectors, host cells are transformed to obtain transformants;

(5) By cultivating the transformants, the human HGF of the present invention can be obtained from the culture supernatant.

Besides, the DNAs containing the nucleotide sequences which code for the human HGF of the present invention can be obtained from the recombinant expression vectors in the transformed cells by way of treatment with restriction enzymes.

The respective processes are in detail described below.

(1) Isolation of mRNA and preparation of cDNA library:

mRNA encoding animal (e.g. rat) or human HGF can be obtained respectively from organs such as liver, kidney, spleen, lung, brain, bone marrow and placenta or blood cells such as leukocyte or megakaryocyte of animals such as rat or human. For example, in accordance with the J. M. Chirgvin et al method as described in Biochemistry, 18, 5294 (1979), said mRNA can be purified by subjecting RNA obtained from the guanidine thiocyanate lysate of animals (e.g. rat) or human organs or blood cells to liquid chromatography of oligo (dT) cellulose column.

Also, various mRNAs of animal cells or tissues such as those of human liver, brain, placenta, leukocyte and so on are available in the market, and the products of Clontech Lab. can be used.

cDNA libraries can be constructed by synthesizing cDNAs using a reverse transcriptase with the above-mentioned mRNA as a template or using polymerase chain reaction (PCR) method, in accordance with, for example, the H. Okayama et al method (Mol. Cell. Biol., 2, 161, 1982 and Mol. Cell. Biol., 3, 280, 1983), the U. Gubler et al method (Gene, 25, 263, 1983) or the M.A. Frohman method (Proc. Natl. Acad. Sci. USA, 85, 8998, 1988) and inserting said cDNAs into plasmids or phage DNAs. Examples of the plasmid vectors into which the cDNAs are inserted include *Escherichia coli*-originated pBR322, pUC18 and pUC19 (Toyobo) and *Bacillus subtilis*-originated pUB110 (Sigma). As the phage vectors into which the cDNAs are inserted, mention is made of λ gt10 and λ gt11 (Toyobo). The above-mentioned examples

of the vectors to be used are not limited and any vectors can be used as long as they are capable of replication and amplification in host cells.

As the methods in which the cDNAs synthesized from mRNA as the template are inserted into plasmids or phage DNAs to produce the cDNA libraries, mention can be made of, for example, the T. Maniatis's method (Molecular Cloning, Cold Spring Harbor Laboratory, 1982, p.239) or the T. V. Hyunh et al method (DNA Cloning: A Practical Approach, 1, 49, 1985). Also, various kinds of cDNA libraries are available in the market like mRNAs and can be purchased from Clontech Corp. and others.

(2) Cloning of cDNA library:

The recombinant expression vectors of plasmids, phage DNAs or so on obtained as cDNA libraries can be maintained in suitable host cells such as *Escherichia coli*. Examples of the *Escherichia coli* as the host cell include *Escherichia coli* NM514, C600 (Stratagene), NM522, JM101 (Pharmacia) and so on. Using the calcium chloride method, the calcium chloride-rubidium chloride method or another method in the case where plasmids are used as the cDNA vector and using the *in vitro* packaging method in the case where phage DNAs are used as the cDNA vector, the recombinant expression vectors can be maintained in host cells which are in advance proliferated (Molecular Cloning, Cold Spring Harbor Laboratory, 1982, p.249).

Oligonucleotides encoding a portion of the amino acid sequences of the hepatocyte growth factors of animal such as rat or human are synthesized. From the thus-obtained transformants, cDNA clones can be screened by using the above-mentioned oligonucleotides labeled with ³²P as a probe by the colony-hybridization method (Gene, 10, 83, 1980), the plaque hybridization method (Science, 196, 180, 1977) or another method. The cloning can be conducted also by the enzyme antibody method (DNA Cloning: A Practical Approach, 1, 49, 1985) with antibodies against the objective polypeptides to give the cDNA clones. The thus-cloned transformants contain the cDNAs having the nucleotide sequences which encode the entire amino acid sequence or a portion of the amino acid sequence of animal- (e.g. rat) or human-originated HGF.

The recombinant DNAs of the plasmid or phage are isolated from the transformants in accordance with the conventional method (Molecular Cloning, Cold Spring Harbor Laboratory, New York, 1982), wherefrom directly as it is or after digested with restriction enzymes, the cDNA nucleotide sequence is determined. The cDNA libraries prepared

from mRNA originated from human organs or blood cells are screened in the same manner with the first-obtained animal- (e.g. rat) or human-originated cDNAs as the probe. The cDNA sequence of animal- such as rat or human-originated HGF can be determined by the Maxam and Gilbert's chemical method (Proc. Natl. Acad. Sci. USA, 74, 560, 1977) or the dideoxy method by Sanger (Proc. Natl. Acad. Sci. USA, 74, 5463, 1977). Furthermore, if desired, another cDNAs are synthesized newly from the above-mentioned mRNA by the primer-extension method (Proc. Natl. Acad. Sci. USA, 76, 731, 1979) using as the primer a portion of the cDNA whose sequence is determined or a synthesized DNA which is a part of the cDNA, and the cloning of the recombinant DNAs of plasmids, phages or so on containing the cDNAs which can be ligated with the cDNA obtained already from the cDNA library can be performed in the same manner as mentioned above. These steps of primer-extension and cloning can be repeated multiple times, if necessary.

(3) Construction of recombinant expression vector for human HGF:

Recombinant expression vectors can be prepared by digesting restriction enzymes cDNAs from several kinds of recombinant vectors of plasmids, phages or so on containing the cDNAs which encode the entire amino acid sequence or portion thereof of human HGF and ligating the cDNAs with the downstream of promoter region of vectors suitable for expression of the human HGF with DNA ligase.

More specifically, for the purpose of permitting the human HGF of the present invention to express efficiently, the recombinant expression vector is constructed, if desired, so that it may contain (1) a promoter, (2) a ribosome binding site, (3) an initiation codon, (4) a DNA containing the nucleotide sequence which encodes the human HGF of the present invention, (5) a termination codon and (6) a terminator in order in the downstream direction of transcription.

As the DNA vectors to be used in the present invention, mention can be made of, for example, *Escherichia coli*-originated plasmid pBR322, pUC18 (Toyobo), *Bacillus subtilis*-originated plasmid pUB110 (Sigma), yeast-originated plasmid pRB15 (ATCC37062), bacteriophage λ gt10, λ gt11, (Stratagene Corp.), Virus SV40 (BRL Corp.), BPV (ATCC VR-703), retrovirus gene-originated vectors and so on, which are not limited, and any DNA vector can be used as long as it is a vector capable of replication and amplification in a host. Particularly, vectors originated from a virus gene such as SV40 are preferable in order to allow the

human HGF of the present invention to express easily.

For example, a recombinant expression vector in which the above-mentioned cloned DNA encoding the human HGF is ligated with the late promoter region of SV40 DNA can be introduced into a simian cell strain called COS cell (Cell, 23, 175, 1981) for expression.

With regard to the promoter and terminator, there is no limitation thereto and any one can be used as long as it corresponds to the host used for expression of the nucleotide sequences which code for the objective human HGF. For example, there can be mentioned *trp* promoter, *lac* promoter and so on as the promoter for the host of *Escherichia coli*, SP01 promoter, SP02 promoter and so on for the host of *Bacillus subtilis*, GAP promoter, PGK promoter and so on for the host of yeast and virus-originated SV40 promoter, HSV1 TK promoter, and metallothionein promoter for the host of animal cells such as mouse fibroblast and Chinese-hamster ovary cell. As the terminator, there can be mentioned, for example, *trp* terminator, *lpp* terminator and so on for the host of *Escherichia coli*, *amyF* terminator for the host of *Bacillus subtilis*, *CYC1* terminator for the host of yeast and SV40 terminator, HSV1 TK terminator and so on for the host of animal cells. These promoters and terminators can be used suitably in combination depending on the host used.

There is no particular limitation to the DNA containing base sequences which code for the human HGF of the present invention as long as the polypeptides prepared from DNA by transcription and translation possess the hepatocyte growth activity, and DNAs having a nucleotide sequence in which said nucleotide sequence is partially substituted, deleted and/or inserted may be used. The translation initiation codon of the DNAs containing the nucleotide sequences which code for the human HGF of the present invention may have ATG, and the translation termination codon may have TAA, TGA or TAG. If desired, more than one termination codon may exist in combination with initiation codon. Initiation codon and termination codons are not limited. It is preferable that the vectors contain at a suitable position one or more ampicillin-resistance gene(s), neomycine-resistance gene(s), DHFR gene(s) and so on which can be a selection marker for the hosts which are transformed by the recombinant expression vectors.

(4) Transformation of host cell and cultivation thereof:

The thus-constructed recombinant expression vectors for the human HGF are introduced into host cells to yield transformants by the competent cell

method (J. Mol. Biol., 53, 154, 1970), the protoplast method (Proc. Natl. Acad. Sci. USA, 75, 1929, 1978), the calcium phosphate method (Science, 221, 551, 1983), the DEAE dextran method (Science, 215, 166, 1982), the electroporation method (Proc. Natl. Acad. Sci. USA, 81, 7161, 1984), the *in vitro* packaging method (Proc. Natl. Acad. Sci. USA, 72, 581, 1975), the virus vector method (Cell, 37, 1053, 1984) or the microinjection method (Exp. Cell. Res., 153, 347, 1984). Herein, use can be made of as the host cell not only *Escherichia coli* as mentioned above but also *Bacillus subtilis*, yeasts, and animal cells. Particularly, it is preferred to use mammalian host cells such as mouse fibroblast C127 (J. Virol., 26, 291, 1978) and chinese hamster ovary cell CHO (Proc. Natl. Acad. Sci. USA, 77, 4216, 1980).

The thus-obtained transformants are cultivated in a culture medium suitable for the host to produce the objective recombinant human HGF. In the culture medium, there may be contained carbon sources, nitrogen sources, inorganic substances, vitamins, sera, medicaments and so forth which are necessary for growth of the transformants. Examples of the culture medium include LB medium (Nissui Pharmaceutical), M9 medium (J. Exp. Mol. Genet., Cold Spring Harbor Laboratory, New York, 1972, p.431) and the like in the case where the host of the transformant is *Escherichia coli*; YEPD medium (Genetic Engineering, vol. 1, Plenum Press, New York, 1979, p. 117) in the case where the host is yeast; and MEM medium, DMEM medium, RPM11640 medium (Nissui Pharmaceutical) and so on containing fetal bovine serum in a proportion of not more than 20%, in the case where the host is an animal cell. The cultivation of the transformants is conducted usually at 20°C - 45°C at pH 5-8, while if necessary aeration and stirring is conducted. When the host is an adherent animal cell, carriers are used such as glass beads, collagen beads or acetyl cellulose hollow fibres. The cultivation can be conducted in a medium of any other medium composition under any other conditions so long as the transformant grows. Thus, said medium composition and cultivation conditions are not limited.

(5) Purification of human HGF:

The recombinant human HGF produced thus in the culture supernatant of the transformants or in the transformants can be separated and purified by known methods such as salt precipitation, solvent precipitation, dialysis, ultrafiltration, gel electrophoresis, gel filtration chromatography, ion exchange chromatography, reverse-phase chromatography, affinity chromatography and so on in combination. Particularly, preferred and effective pu-

rification methods are a combination of salt precipitation with ammonium sulfate, S-Sepharose ion exchange chromatography, heparin-Sepharose affinity chromatography and phenyl-Sepharose reverse-phase chromatography and a combination of salt precipitation with ammonium sulfate, S-Sepharose ion exchange chromatography and anti HGF antibody-Sepharose affinity chromatography, etc.

The recombinant human HGF obtained by the above-mentioned methods exhibits an excellent growth promoting activity for hepatocytes in the same manner as with a rat liver- or rat platelet-originated HGF.

It is also possible to extract, isolate and purify HGF from animal tissues including those of humans and cultured animal cells, and methods therefor include conventional extraction, isolation and purification for proteins. For example, organic solvent precipitation with ethanol or acetone, salt precipitation with ammonium sulfate, etc., dialysis, ultrafiltration, ion-exchange chromatography, gel filtration chromatography, reverse-phase chromatography, hydrophobic chromatography and affinity chromatography may be used in combination. Particularly preferred are combinations of S-Sepharose chromatography, heparin-Sepharose chromatography, phenyl-Sepharose chromatography, antibody affinity chromatography, C4 reverse-phase chromatography and pigment affinity chromatography, which are effective methods of isolation and purification.

Any HGF can be used for the present invention as long as it has sufficient hepatocyte-growing activity and fibroblast growth-inhibitory activity which are effective for the treatment of hepatocirrhosis, even if part of its amino acid sequence is deleted or substituted, or other amino acid sequence is partly inserted, or sugar is deleted or substituted in the same manner.

The HGF which is an active ingredient in the present invention shows excellent activities for hepatocyte growth and suppression of fibroblast growth in mammals such as human, cow, horse, rat, sheep and so on, and is an efficacious agent for hepatocirrhosis for all mammals.

The therapeutic agents of the invention are generally formed into injections containing HGF solely or combinedly with carriers, etc. known per se. For example, injections can be prepared by dissolving HGF in suitable buffers, followed by sterilization by filtration through a filter.

The therapeutic agents for hepatocirrhosis of the invention may contain other additives such as stabilizers, excipients, dissolution-promoters, adsorption-preventors and antioxidants, and examples thereof include, for example, sugars such as mannitol and glucose, amino acids such as glycine, alanine, lysine and arginine, proteins such as al-

bumin, alcohols such as ethylene glycol and glycerol, hydrophilic polymers such as polyethylene glycol, inorganic salts such as NaCl, organic salts such as sodium citrate, surfactants such as Polysorbate 80 and reducing agents containing sulfur, which may be used alone or in combination.

The liquid preparations are preferably stored by cryopreservation or after removal of water content by freeze-drying or vacuum drying. An aqueous solution containing HGF may be subjected to salt precipitation or solvent precipitation for precipitation of the factor, after which the obtained precipitate is dried and stored.

The therapeutic agents for hepatocirrhosis of the invention can be generally administered intravenously, intra-arterially or subcutaneously. The dose amount ranges from 0.01 mg to 100 mg by the amount of HGF, which can be administered singly or in several times divided doses.

The therapeutic agents for hepatocirrhosis of the invention contain HGF as an active ingredient. The HGF not only suppresses fiber formation by the fibroblast proliferation-inhibitory activity but simultaneously stimulates hepatocyte proliferation, exhibiting two physiological activities of specific stimulation of hepatocyte proliferation and specific inhibition of hepatic nonparenchymal fibroblast proliferation, and thus, is considered to be effective for preventing the transition from chronic hepatitis to hepatocirrhosis and progress of hepatocirrhosis, as well as for the treatment of considerably advanced hepatocirrhosis. Thus, HGF is highly useful as a therapeutic agent for hepatocirrhosis for which there have been no dependable treatment methods, and also for hepatic diseases such as hepatitis.

The present invention is hereinbelow described in detail by illustrating working examples, reference examples and experimental examples to which the invention is not limited.

Reference Example 1

[Assay of HGF activity]

HGF activity was assayed in the following manner in accordance with the method described in Proc. Natl. Acad. Sci. USA, 80, 7229 (1983). Hepatocytes were isolated and purified from Wistar rats by the collagenase reflux method. The obtained rat hepatocytes were suspended in the Williams E medium (Flow-Laboratory Corp.) to which 5% fetal bovine sera, 2×10^{-9} M insulin and 2×10^{-9} M dexamethasone had been added, and the suspension was sown on a 24-well multiplate in the concentration of 1.25×10^5 cells per well. After the cells were cultured in the presence of 5% CO₂, 30% O₂ and 65% N₂ at 37°C for 20 hours, the

medium was replaced with Williams E medium containing 0.1 µg/ml of aprotinin, and at the same time, the prescribed amount of test samples were added. Fifteen hours later, 15 µCi/ml of ¹²⁵I-deoxyuridine was added thereto (10 µl/well). To the control, 5 µg/ml of aphidicolin was added 15 minutes before the addition of ¹²⁵I-deoxyuridine. The cells were cultured for further 6 hours and labeled with ¹²⁵I. The cells were washed twice with PBS at pH 7.4 and then immobilized with a cold 10% trichloroacetic acid (TCA) solution. The cells were solubilized with 1N NaOH in an amount of 0.5 ml per well, and the radioactivity was assayed by a gamma counter. After the assay of radioactivity, a portion of the sample was measured for the amount of the protein in accordance with the Lowry method (J. Biol. Chem., 193, 265, 1951). The amount of ¹²⁵I incorporated into the hepatocytes with the addition of the test sample was measured as the difference in count from that of the control, from which the amount per 1 mg of the rat hepatocyte protein was estimated and taken as the DNA synthetic activity (dpm/mg protein). The HGF activity of the test sample was indicated on the basis of the definition that the activity corresponding to the 50% synthetic activity of the hepatocyte DNA in the same test with the use of 10 ng/ml of epithelial cell growth factor (EGF) being 1 unit.

Reference Example 2

[Assay of hepatic nonparenchymal fibroblast proliferation-inhibitory activity]

A crude cell dispersion was prepared by perfusing liver from young mature rat (weighing about 180 g) with collagenase, which was then subjected to low speed centrifugation at 50 × g for 1 minute to isolate the supernatant from hepatocytes precipitated.

The supernatant was subjected to centrifugation at 100 × g for 3 minutes to completely precipitate parenchymal hepatocytes, followed by centrifugation at 350 × g for 3 minutes to precipitate nonparenchymal hepatocytes. The thus-obtained nonparenchymal hepatocytes containing practically no parenchymal hepatocytes were suspended in an RPMI 1640 medium containing 10% bovine serum at a concentration of about 260,000 cells/ml, and cultivated in a 12-well multiplate at 1 ml per well. After 3 days' cultivation, the medium was replaced with a fresh RPMI 1640 medium containing 10% fetal calf serum and cultivation was continued. On day 7 from initiation of the cultivation, human or rat liver-originated HGF was added at a concentration of from 1 ng to 5 ng/ml. Twenty-four hours later, 2.5 µCi of [³H]-thymidine was added to each well, followed by further cul-

tivation for 12 hours. The medium was removed and cells were washed well with PBS. A gender solution (1 ml) was added to each well to immobilize the cells. Twenty minutes later, the gender solution was removed, and the cells were washed with PBS and coated with an X-ray photosensitive emulsion [emulsion type NR-M2 (Konishiroku Corp.)] in a darkroom. After 6 days' exposure at 4 °C, the cells were fixed and developed to yield an autoradiogram. After developing and fixation, cytoplasm was stained with eosin and photographed. Labeling index was estimated by counting nonparenchymal hepatocytes whose nuclei were labeled.

Reference Example 3

[Production of hepatocyte growth factor from liver]

Carbon tetrachloride (0.2% body weight of rat) was intraperitoneally administered, and 30 hours later, the rat liver was excised. The liver was pulverized by a whirling blender, after which it was centrifuged at 10,000 rpm for 20 minutes with a Hitachi 20 PR-52 cooling centrifuge to give the supernatant. The supernatant was dialyzed with a mixed solution of 50 mM tris hydrochloric acid (pH 8.5), 0.15 M NaCl, 10 mM HEPES, 2 mM CaCl₂ and 0.01% Tween 80 at 4 °C for a whole day. The dialyzed solution was poured onto an S-Sepharose (FF, Pharmacia) column equilibrated with dialysing fluid, and after washing, it was eluted with the concentration gradient of NaCl. The hepatocyte growth factor was eluted near the NaCl concentration of 0.7 M. The hepatocyte growth factor was then purified by Blue Tris acryl M (IBF Corp.) chromatography. Elution was conducted with the concentration gradient of arginine, and the hepatocyte growth factor was eluted at an arginine concentration of near 0.25 M. The obtained fraction was then purified by heparin-Sepharose (Pharmacia) chromatography. Elution was conducted with the concentration gradient of NaCl, and the hepatocyte growth factor was eluted at an NaCl concentration of about 1 M, which was then purified by phenyl 5PW (Toso Corp.) chromatography. Elution was conducted with the concentration decrease gradient of NaCl and the concentration increase gradient of ethylene glycol, and the hepatocyte growth factor was obtained by 10 µg per livers from 100 rats. The purified hepatocyte growth factor showed a band of 70,000 to 90,000 molecular weight under nonreducing conditions and after reduction, it showed two bands of α-chain of about 70,000 molecular weight and β-chain of about 30,000 molecular weight by SDS-PAGE electrophoresis. To the obtained hepatocyte growth factor was added 0.25% BSA (bovine serum albumin),

which was then dialyzed with PBS and used for experiment.

Reference Example 4

[Production of human hepatocyte growth factor using C127 cells as a host by genetic recombination]

Mouse C127 cells transformed by a gene coding for the amino acid sequence of human hepatocyte growth factor were cultivated, and human hepatocyte growth factor was obtained from the culture supernatant thereof. The processes are described in the following.

Clone HAC19 and HBC25 coding for the amino acid sequence of human hepatocyte growth factor were obtained by screening cDNA libraries constructed from human liver mRNAs.

DNA from the HAC19 and the HBC25 were digested with BamHI and ScaI, and ScaI and PstI, respectively. The thus-obtained two DNA fragments were ligated with Blue Script KSII at BamHI and PstI sites to obtain pBS[hHGFII] (FERM P-11050 deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan). The pBS[hHGFII] was digested with XbaI, SalI and NaeI, and given blunt ends by T4 DNA polymerase, after which an about 3 Kb DNA fragment coding for human hepatocyte growth factor was inserted at EcoRV site of an expression vector pBPMT prepared with bovine papilloma virus DNA as a vector, to give pBPMT[hHGFII]. The thus-obtained hepatocyte growth factor expression vector pBPMT[hHGFII] was used to transform mouse C127 cells by the calcium phosphate method. Selection of the transformants was conducted by growth thereof in a medium containing G418. The cell line BPH89 showing high producibility of a hepatocyte growth factor was selected from among the obtained transformants. After the BPH89 cells were grown in a medium supplemented with fetal calf serum, the medium was replaced every 2 days to permit production of a hepatocyte growth factor. The objective hepatocyte growth factor was purified from the culture medium by a modification of the purification method as described in Reference Example 3. It was confirmed that the purified hepatocyte growth factor showed a single band of about 80,000 daltons molecular weight under non-reducing conditions and two bands of α -chain of about 70,000 daltons molecular weight and β -chain of about 30,000 daltons molecular weight under reducing conditions by SDS polyacrylamide gel electrophoresis. To the obtained hepatocyte growth factor was added 0.25% BSA and after dialysis with PBS, it was used for experiment.

Experimental Example

[Effect of HGF on hepatocytes in primary culture and on hepatic nonparenchymal fibroblasts in primary culture]

The purified human recombinant hepatocyte growth factor as prepared according to Reference Example 4 was added to primary mature rat hepatocyte culture so that the concentration became 1-5 ng/ml according to the method as described in Reference Example 1, and DNA synthesis was measured. Further, the factor was added to rat nonparenchymal hepatocytes at 1-5 ng/ml as described in Reference Example 2, and labeling index was calculated. The results are summarized in a graph as Fig. 1. The recombinant human hepatocyte growth factor promoted growth of hepatocytes dose-dependently, whereas markedly inhibited growth of nonparenchymal hepatocytes. That is, the factor acts completely reversely on parenchymal cells and nonparenchymal cells constituting a liver.

Example 1

An aqueous solution is prepared aseptically by adding 1 mg of a hepatocyte growth factor and 100 mg of human serum albumin to 100 ml of 0.02 M phosphate buffer (pH 7.4) containing 0.15 M NaCl and 0.01% Polysorbate 80, and filled in a vial at 1 ml per vial, followed by lyophilization and sealing. Injectable distilled water is filled in an ampoule at 1 ml each for dissolution.

Example 2

An aqueous solution is prepared aseptically by adding 1 mg of a hepatocyte growth factor and 100 mg of human serum albumin to 100 ml of 0.02 M phosphate buffer (pH 7.4) containing 0.15 M NaCl and 0.01% Polysorbate 80, and aseptically filled in an ampoule at 1 ml per ampoule, followed by melt-sealing.

Example 3

A solution is prepared aseptically by adding 1 mg of a hepatocyte growth factor, 1 g of mannitol and 10 mg of Polysorbate 80 to 100 ml of physiological saline and filled in a vial at 1 ml per vial, which is then lyophilized and sealed.

Example 4

An aqueous solution containing 1 part by weight of a hepatocyte growth factor, 50 parts by weight of human serum albumin and 100,000 parts

by weight of injectable distilled water is prepared aseptically and filled in a vial, which is then lyophilized and sealed.

Example 5

A solution is prepared aseptically by adding 1 mg of a hepatocyte growth factor, 10 mg of Polysorbate 80, 2 g of glycine and 2 g of sorbitol to 100 ml of injectable distilled water, and filled in a vial at 1 ml per vial, which is then lyophilized and sealed.

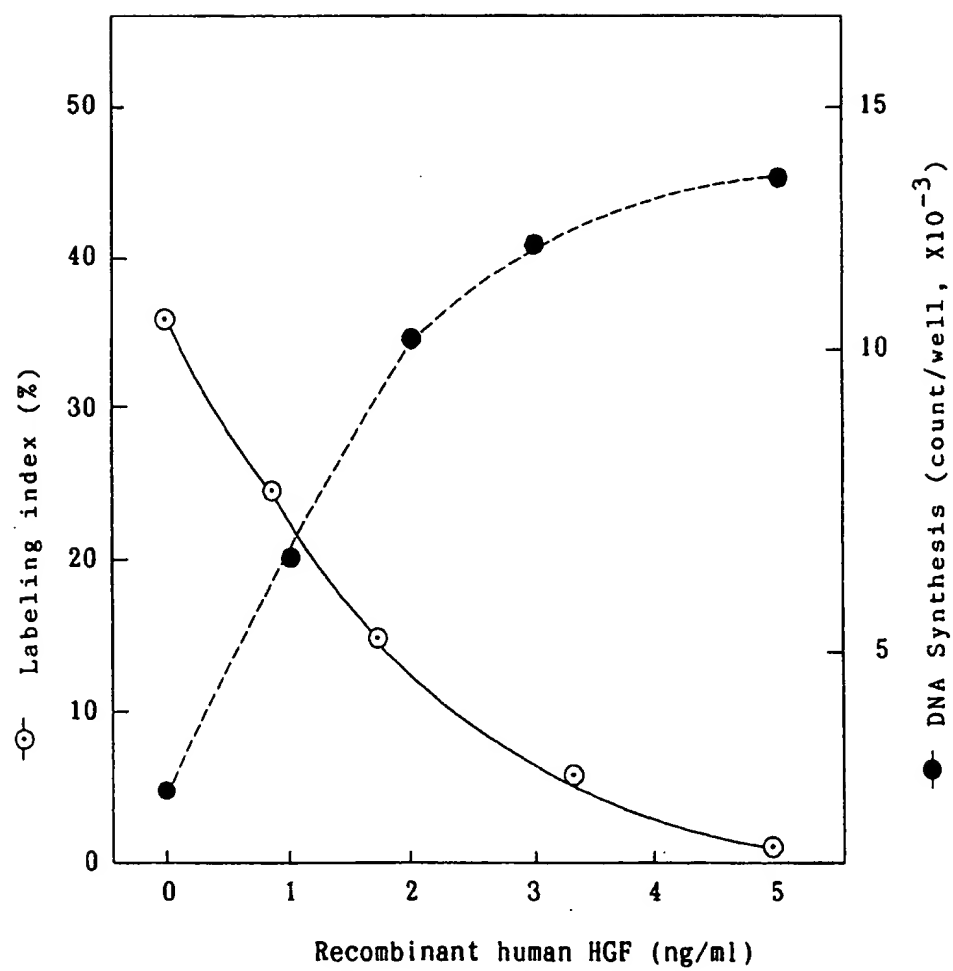
Claims

1. Use of a hepatocyte growth factor for the preparation of a therapeutic agent for the treatment of hepatocirrhosis. 15
2. Use as claimed in Claim 1, wherein the hepatocyte growth factor is a polypeptide having activities of stimulating hepatocyte proliferation and inhibiting proliferation of hepatic mesenchymal cells and fibroblast. 20
3. Use as claimed in Claim 1, wherein the hepatocyte growth factor is originated from animal tissues including those of human. 25
4. Use as claimed in Claim 1, wherein the hepatocyte growth factor is obtained by phenotypic expression of a gene coding for the hepatocyte growth factor in host cells. 30
5. Use as claimed in Claim 4, wherein the host cells are of a species selected from the group consisting of *Escherichia coli*, *Bacillus subtilis*, yeasts, filamentous fungi, plant cells and animal cells. 35
6. Use as claimed in Claim 1, wherein the hepatocyte growth factor is a polypeptide having a molecular weight of 70,000 to 90,000 by nonreduction polyacrylamide gel electrophoresis, which takes the heterodimer structure comprising α -chain of 60,000 to 75,000 molecular weight and β -chain of 30,000 to 40,000 molecular weight under reducing conditions. 40 45
7. Use as claimed in Claim 1, wherein a therapeutically effective amount of the hepatocyte growth factor is contained in admixture with a pharmaceutically acceptable carrier. 50
8. Use as claimed in Claim 1, wherein the therapeutic agent is suitable for parenteral administration. 55

9. A composition comprising a therapeutically effective amount of a hepatocyte growth factor in admixture with a pharmaceutically acceptable carrier.

10. The composition according to Claim 9 suitable for parenteral administration.

Fig - 1





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EUROPEAN SEARCH REPORT

Application Number

EP 91 10 7405

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X,D	NATURE. vol. 342, no. 6248, 23 November 1989, LONDON GB pages 440 - 443; NAKAMURA T. et al: "Molecular cloning and expression of human hepatocyte growth factor" * page 443, column 1 - column 2 * - - -	1-10	C 07 K 15/00 A 61 K 37/02
X	PATENT ABSTRACTS OF JAPAN vol. 12, no. 230 (C-508)(3077) 29 June 1988, & JP-A-63 22526 (SHUJI HASHIMOTO) 30 January 1988, * the whole document * - - -	1-10	
X	PATENT ABSTRACTS OF JAPAN vol. 11, no. 232 (C-437)(2679) 29 July 1987, & JP-A-62 45530 (OTSUKA PHARMACEUTICAL) 27 February 1987, * the whole document * - - -	1-10	
X,P	PATENT ABSTRACTS OF JAPAN vol. 15, no. 60 (C-805)(4588) 13 February 1991, & JP-A-2 288899 (TOYOBO) 28 November 1990, * the whole document * - - -	1-10	
A	PATENT ABSTRACTS OF JAPAN vol. 9, no. 175 (C-292)(1898) 19 July 1985, & JP-A-60 45534 (OTSUKA SEIYAKU) 12 March 1985, * the whole document * - - -	1-10	TECHNICAL FIELDS SEARCHED (Int. Cl.5) C 07 K A 61 K
X,P	EP-A-0 412 557 (MITSUBISHI KASEI) * page 2, lines 41 - 50 * - - - - -	1-10	
The present search report has been drawn up for all claims			
Place of search Berlin		Date of completion of search 23 July 91	Examiner AVEDIKIAN P.F.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			